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# ANNUAL REVIEW OF PHYSIOLOGY

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## PREFACE

It is not without a sense of misgiving that the present Review is introduced to a world already burdened with scientific publications. We realize clearly that the number of journals and books which libraries, institutions, and individual physiologists feel obliged to purchase is not far short of overwhelming. Nevertheless, we offer no apologies for launching the *Annual Review of Physiology*. We are confident that it will fill a distinctive and useful rôle in providing the reader with a comprehensive survey of the year's research in physiology.

It may be that even a cursory inspection of the present volume will raise some doubt in the reader's mind as to whether we have used the adjective "comprehensive" advisedly, for in many instances the reader may be distressed to find that the reviews are more restrictive than inclusive. We fear, however, that this is inevitable. The number of investigators in Physiology is great, and the volume of work published is of such dimensions that the reviewer who would do justice to all papers published in his field in the period under review is baffled at every turn. Confronted by this problem, we have chosen to advise the authors of the reviews to attempt, rather, a critical appraisal of the contemporary field—an analysis and interpretation of the most significant contributions. This has been done in the full realization that certain topics would have to be put aside quite arbitrarily. However, we are impressed by the thought that reviewers in later volumes, if only because of their different research interests, will give to the topics under review an entirely different type of emphasis from that which they may receive in the present volume. It is also evident that several fields of investigation which may now be rather quiescent will burst into fresh activity and assume a commanding interest within the next few years. For the progress of discovery in the experimental sciences is not characterized by a constant and uniform rate of accretion of knowledge, but is distinguished always by sudden advances in seemingly unrelated fields as an occasional epochal investigation liberates a great flood of new research.

It is our hope that this new Review will supplement the invaluable service which has been rendered for many years by *Physiologi-*

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*cal Reviews* and the *Ergebnisse der Physiologie*. We have not thought of publishing exhaustive treatments of selected topics such as may be found in these journals. The *Annual Review of Physiology* will have a somewhat different function and will be of value to the reader, not because of its depth of penetration into stated subjects, but because of its breadth, through year by year resumés of the significant work in the entire field of physiology.

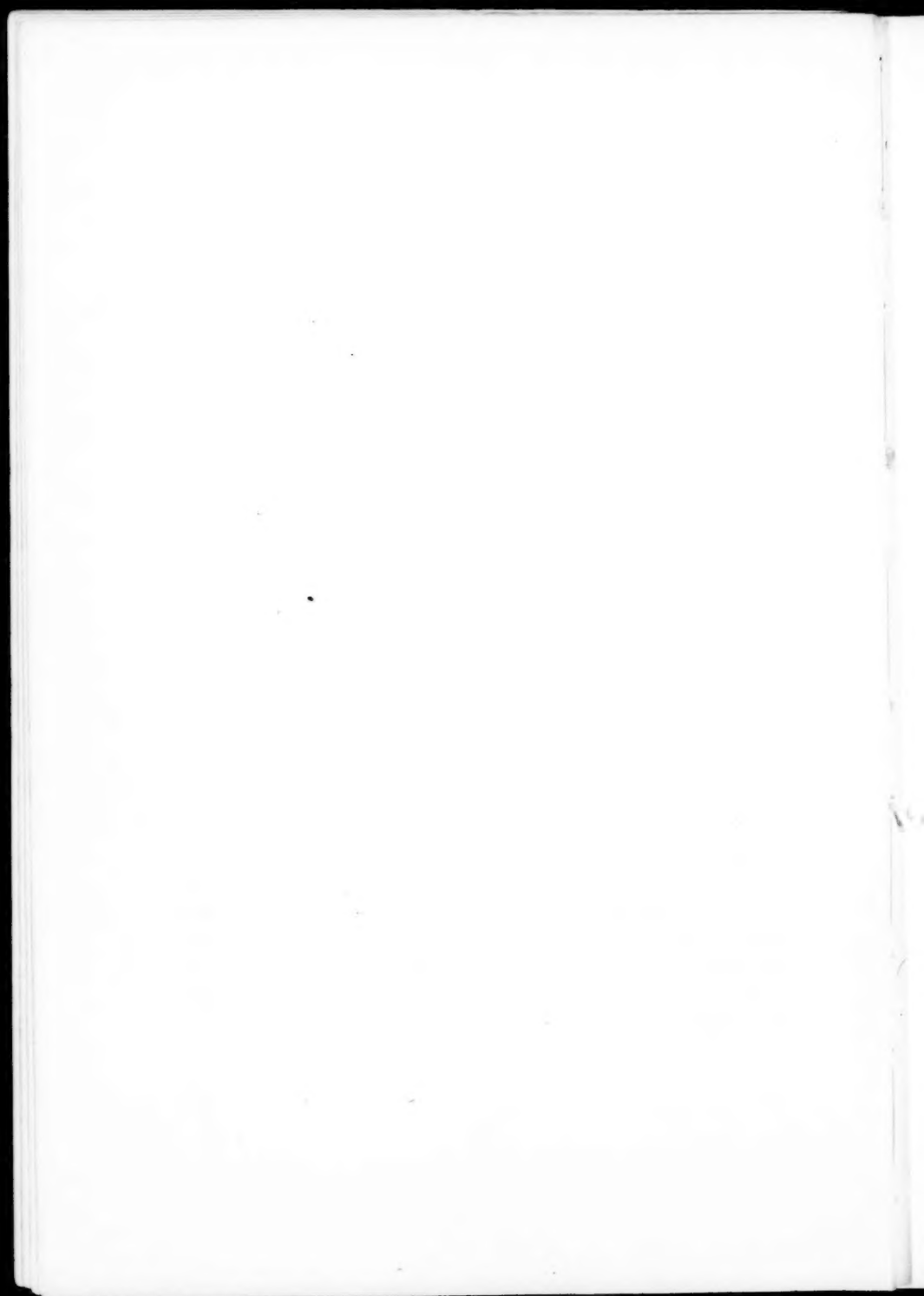
To those who have been good enough to extend support, either through endorsement of the enterprise, advice, and encouragement, or through direct collaboration in authorship, we wish to express our thanks. The reviewers have been beset with innumerable difficulties of which perhaps the greatest has been the necessity of sharply restricting the scope of their reviews and of compressing the subject matter into a severely limited space.

To our printers, the George Banta Publishing Co., we are indebted for splendid cooperation throughout.

A.J.C.	J.M.L.
J.F.F.	F.C.M.
M.H.J.	W.J.M.

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## PERMEABILITY\*

By M. H. JACOBS

*University of Pennsylvania, Philadelphia, Pennsylvania*

This review forms a continuation of the similar one by Collander (1), the period covered being the latter part of the year 1936, all of 1937 and somewhat more than the first half of 1938. For convenience the term "permeability" is here treated in the customary rather loose sense as a measure of the apparent ease of passage of water and dissolved substances through membranes or other regions, whatever the mechanism of the passage may be. It should be noted, however, that mere rate of passage is a measure of permeability only under properly standardized conditions. Teorell (2) has rightly emphasized the distinction between permeability and driving forces of different sorts, and Brooks (3) has also called attention to the danger of confusing rate factors, properly associated with permeability, with equilibrium factors which are not. A concrete example of the way in which a change in permeability may falsely seem to be produced by a change in equilibrium conditions is furnished by the behavior of the erythrocyte in the absence and presence of low concentrations of electrolytes (4).

During the period under consideration an unusually large number of reviews and general articles dealing with permeability and related topics have appeared. The most extensive of these is the large volume by Gellhorn & Régnier (5). Especially rich in references to the recent literature is the review by Wilbrandt (6). That by Collander (1), mentioned above, covers the period 1934 to 1936, and a similar one by Teorell (2) the period 1935 to 1937. One by Höber (7) deals very thoroughly with the permeability of artificial membranes, and another by Ferry (8) with the related problem of ultrafiltration. In the field of plant physiology several reviews by Osterhout (9, 10, 11, 12), though not devoted exclusively to permeability, contain much interesting material in this field, as does also one by Hoagland (13). Perhaps the most important general summary of recent work is represented by the contributions of 32 different workers to the Symposium on the Properties and Functions of Membranes, Natural and Artificial, held by the Faraday Society in April, 1937. It is impossible to list all the

\* Received October 17, 1938.

topics covered in the published report of this meeting (14), but they include, among others having to do specifically with permeability, such subjects as permeability of animal membranes (15), permeability of capillaries (16), permeability of erythrocytes (17), permeability of plant cells (18), etc.

*Permeability to water.*—According to several earlier investigators the egg of the trout, though somewhat permeable to water when first laid, soon becomes impermeable and retains this rather unusual property for a considerable time. Krogh & Ussing (19) have taken advantage of the peculiarities of deuterium oxide to investigate this question further, and have confirmed the general conclusions of previous workers. They find by analysis both of the water surrounding the egg and of that within it at the end of the experiment that in eggs fresh from the oviduct there is at first some permeability to deuterium oxide, but that this disappears within a few hours. In fertilized eggs the impermeability to deuterium oxide continues for many days, but is lost by the time the eyes have developed. The egg is, however, at all times permeable to oxygen.

Recent workers have added several new types of cells to those for which quantitative data on osmotic movements of water are now available. Shapiro & Parpart (20), studying the osmotic volume changes of leucocytes of man and the rabbit by a photoelectric method, have obtained permeability constants for water intermediate in magnitude between those previously reported for mammalian erythrocytes on the one hand and for most plant and animal cells on the other. Kitching (21) has studied the rate of swelling of certain Infusoria after paralysis of the contractile vacuole by potassium cyanide and has reported for *Zoothamnion* a permeability constant of 0.125 to 0.25  $\mu^3$  per  $\mu^2$  per atm. per minute.

Adolph (22) has made quantitative measurements of the rate of passage of water across the body wall of the marine worm *Phascolosoma* both after placing the worms in diluted and concentrated sea water and after the introduction of various concentrations of sea water into the body cavity. For endosmosis a high constant was obtained which did not change with time; for exosmosis a very much lower one which increased with time, though never attaining nearly the value found in the swelling experiments. No evidence could be obtained that non-electrolytes, such as ethylene glycol



or urea, penetrate the body wall at unequal rates in the two directions. In studying the effects of alcohols on permeability, Saubert (23) also made quantitative comparisons of the relative rates of exosmosis and endosmosis under standardized conditions in cells of the plant *Chara*, and found the former process to be consistently more rapid than the latter.

The much-discussed question of possible differences in the rate of movement of water across frog skin in the two directions, which doubtless involves other factors than permeability in the strict sense, is still in dispute. Huf (24, 25, 26) has given support to the view of earlier workers that osmosis across frog skin is of an irreciprocal nature but Rubinstein & Miskinowa (27) and Jurišić (28) have reached the opposite conclusion. In connection with all such studies it is important, as has been emphasized by Krogh (15), to remember the osmotic complications introduced by the active transport of ions across frog skin from outside inward (see page 14).

Experiments on what has been called the permeability of the roots of higher plants to water also present certain complications. Brewig (29, 30) exposed the roots of *Vicia faba* to sugar solutions of different concentrations in a "micro-potetometer." The rates of movement of water both inward and outward when plotted against the concentration of the sugar solution were found to fall on the same straight line, as would be expected in a simple osmotic system. When, however, the rate of transpiration of the plant was artificially increased, there was a great increase not only in the rate of endosmosis but of exosmosis as well, which Brewig interprets as indicating an increase in the permeability of the root, set up by a stimulus transmitted in some unknown manner from the shoot. A simpler possibility would seem to be that any increase in convection currents within the root would steepen the osmotic gradients in the regions in which the exchanges occur.

*Permeability to non-electrolytes.*—A very valuable collection of permeability constants for plant cells for non-electrolytes is given by Elo (31), who in addition to his own determinations for a number of types of cells not previously investigated has collected from the literature all the similar data available, so that a fairly exact comparison is now possible of the permeability of 29 different kinds of plant cells to all or most of the following substances: acetamide, ethylene glycol, methyl urea, urea, glycerol, malonamide

and erythritol. From such data it is possible to construct "permeability series" in the sense of Höfler and Stiegler. This question has been discussed in a recent paper by Höfler (32). One generalization that emerges from comparative studies of this sort is that among plant cells there may be distinguished a "urea type" of permeability and a "glycerol type." Between the two extremes, however, all possible gradations seem to exist. Hofmeister (33) has shown that certain cells of *Ranunculus repens* may change from the "urea type" to the "glycerol type" during their normal life history.

Several years ago Zehetner found that when placed in aqueous solutions of alcohol some plant protoplasts first swell and then shrink (expansion type), while others first shrink and then swell (contraction type). These differences were attributed to differences in the relative permeabilities of the cells to water and to alcohol respectively. Ruhland, Ullrich & Endo (34) have continued these studies and have shown that such a distinction is difficult to make, since the behavior of the same kind of cell can readily be reversed by such factors as the presence or absence of electrolytes, the degree of plasmolysis, or the temperature.

Among animal cells, specific peculiarities in permeability are particularly well illustrated by the erythrocyte. Comparisons of these cells from different classes of vertebrates (35) show very striking examples of the "urea type" and the "glycerol type" of permeability mentioned above. At the one extreme, the erythrocytes of the mammals are more permeable to urea than any other known cells and show highly characteristic specific differences in their permeability to glycerol. At the other extreme, those of many birds show a low permeability to urea but a permeability to glycerol almost or quite as great as that to ethylene glycol—a condition apparently not yet reported for any other kind of cell. As contrasted with the birds, the reptiles seem to be characterized by erythrocytes having an unusually low permeability to glycerol but a fairly high permeability to urea. Within a single class of vertebrates the erythrocytes even of two species as closely related as the albino rat and the albino mouse can readily be distinguished from each other by permeability characters. Jacobs, Glassman & Parpart (36) have reported twelve such distinguishing characters, which include not merely ordinary differences in permeability to certain selected substances but other characteristic effects on permeability of temperature, pH, traces of copper, etc.

Among the apparently specific peculiarities of the human erythrocyte is a permeability to glucose which it has long been believed to share with those of the higher apes but not with those of the common laboratory animals. Doubt was cast upon this distinction by Olmsted (37) who reported that the presence of relatively high concentrations of glucose in human erythrocytes is not a normal condition but results from the use of anticoagulants. The subject has, however, been reinvestigated by several other workers (38, 39, 40), who are unanimous in disagreeing with Olmsted's conclusions and in supporting the earlier view. Wilbrandt (41) has also investigated the permeability of the erythrocytes of several species of mammals to a series of different sugars, both hexoses and pentoses, and has found a high degree of specificity in the permeability series thus obtained.

Not only can specific differences in the permeability of erythrocytes to non-electrolytes be demonstrated, but individual differences have also been reported. Ørskov (42), for example, has found in individual rabbits rather large differences in permeability to glycerol, though not to thiourea. Great increases in permeability to glycerol but not to thiourea were also observed in rabbits after hemorrhage, but were not obtained in dogs. Bang & Ørskov (43) have found very considerable individual differences in the permeability of human erythrocytes to glucose as well as to certain other substances. In particular, in pernicious anemia they have reported as much as a four-fold increase in permeability to glucose, which, however, disappears with treatment. Of further general interest in this paper are the high  $Q_{10}$  values of 5.0 to 6.25 reported for the penetration of glucose into the human erythrocyte and the pronounced effect on the process of relatively small changes in the concentration of the penetrating substance.

Among the studies of the permeability to non-electrolytes of tissues rather than of individual cells, one of particular interest is that of Höber & Höber (44) on the rate of absorption of various organic compounds in the intestine of the rat. Though the absorption of ions and certain substances of physiological importance is generally acknowledged to be a process of considerable complexity, the behavior of most of the compounds here studied proved to be comparatively simple and in accordance with principles generally applicable to single cells. Thus with a series of polyhydric alcohols the rate of absorption decreased with increasing molecular volume

and became practically zero with mannitol. The same behavior was observed with a series of amides, though for approximately equal molecular volumes amides of low lipoid-solubility were more slowly absorbed than the alcohols. Among the amides themselves those of relatively high lipoid-solubility tended to be more rapidly absorbed than others of smaller molecular volume but low lipoid-solubility. Of all the substances studied only the amino acids failed to show the simple quantitative relation between the concentration of the solution and the rate of absorption that would be expected of simple diffusion processes. The latter compounds were also found to be absorbed more rapidly than other substances of comparable molecular volume. According to Laszt (45) the rate of absorption of glycocoll, but not of certain other amino acids, is reduced to about half by extirpation of the adrenals.

An interesting example of the differences between intestinal absorption by simple diffusion and by a method that seems to involve other factors as well is furnished by the behavior of xylose on the one hand and glucose on the other, as recently reported by Verzář and his collaborators. The rate of absorption of xylose seems to be little affected by extirpation of the adrenals (46, 47, 48) or the hypophysis (49), while that of glucose under the same conditions is greatly reduced. In the rat, galactose behaves like glucose, and mannose, sorbose and arabinose like xylose (47). Furthermore, glucose is absorbed more readily in the upper than in the lower part of the small intestine and its rate of absorption is greatly reduced by cooling; xylose does not show these differences (50).

Another study involving tissue permeability to non-electrolytes is that of Haywood & Höber (51), who found that whereas the liver of the frog strongly concentrates the lipoid-insoluble dye, eriocyanin A, supplied to it in a perfusion fluid, other substances such as xylose, lactose, inulin and  $Mg^{++}$  appear in the secretion without a significant change in their concentration. To such substances they believe that the liver cells behave like a passive filter, the pore size of which is large enough to allow the passage of the inulin molecule. An upper limit to the pore size is, however, set by molecules of soluble starch which fail to appear in the secretion under the conditions of the experiments.

*Permeability to ions.*—Of all cells whose permeability to ions has been accurately studied, the erythrocyte (with the possible

exception of the leucocyte) seems to be unique in possessing a free permeability to at least many anions. A recent study dealing with the rate of exchange of chloride and bicarbonate ions between the erythrocyte and its surroundings is that of Luckner & Lo-Sing (52), who measured changes in the chloride concentration of the external medium by means of a chlorine electrode. They found that the exchange is practically complete in two or three seconds. However, the true time must be considerably less than this figure, since control experiments indicate a rather large lag due to the time required for the attainment of steady conditions at the electrode. By the same method Timm (53) has studied the rate of exchange of chloride for sulphate and for the ions of lactic, acetoacetic,  $\beta$ -hydroxybutyric, oxalic and phosphoric acids. All these ions enter the erythrocyte in exchange for chloride with considerable readiness, though much more slowly than bicarbonate. The rate of exchange seems to be increased in hypotonic and decreased in hypertonic solutions. The exchange of sulphate for univalent anions can also readily be followed by means of the reversible osmotic volume changes of the cells that occur when single bivalent ions replace pairs of univalent ions or vice versa (54). It may be noted that the temperature coefficient for the penetration of the sulphate ion, like that for inorganic phosphate studied by Halpern (55), is large, but this fact in itself does not necessarily require a chemical explanation (56).

The question of a possible permeability of the erythrocyte to cations under more or less normal conditions is still in dispute. The ammonium ion, whose ready penetration is sometimes assumed, probably does not in itself differ from other cations, its apparent ease of entrance being due to its equilibrium with free ammonia (57). As to other cations, there seems to be no doubt that injurious agents of various sorts may cause a leakage, especially of potassium, from the erythrocyte. Among such agents which have recently been investigated are low concentrations of lead (58), silver, and copper (59), all of which under appropriate conditions cause a rapid loss of potassium from red cells rich in this ion. In view of this behavior, until mercury can be shown to be entirely harmless, results obtained with blood which has previously been in intimate contact with it should be looked upon with some suspicion.

Another factor which has recently been shown to be effective

under certain circumstances in causing an escape of potassium from erythrocytes is a hypotonicity of the surrounding solution. According to Davson (60) a fairly rapid loss of potassium from erythrocytes of several species occurs in hypotonic sodium chloride solutions at 40°C., though little loss, except perhaps that attributable to centrifuging, occurs in similar solutions at temperatures of 20°C. or lower. Such losses may also be inferred from studies of hemolysis (61). The fact that in hypotonic solutions potassium ceases to be lost long before its concentrations inside and outside the cells have become equal is attributed by Davson to a return of the swollen cells to volumes at which they are no longer permeable to cations. At this point, however, further dilution of the solution is able to cause a further loss of potassium. Henriques & Ørskov (62) have also obtained evidence of a loss of potassium from erythrocytes *in vivo* after the introduction of distilled water into the circulation of rabbits. The work of these investigators as well as that of Davson suggests that while an increase in permeability to sodium may accompany an increase to potassium, the former is far less striking, as might be expected from the known properties of the two ions.

A factor, possibly of considerable practical importance in all work with erythrocytes, is the effect of centrifuging. Crabtree & Maizels (63), using as a control a rapidly sedimenting human blood, concluded that centrifuging such blood for ten minutes at 4,500 and 10,000 r.p.m. causes no very appreciable loss of potassium. Davson & Danielli (59) by a similar method found a considerable loss from erythrocytes of the ox and the dogfish, though in otherwise normal cells the amount was not strikingly great. It would seem, however, from data to be found in earlier papers by Davson and elsewhere that while centrifuging normal cells may be a fairly harmless procedure, the same is by no means necessarily the case with cells that have been subjected to slight injuries of different sorts, in themselves perhaps insufficient to cause leakage. It is at least conceivable that some of the anomalous results to be found in the literature may be due to the fact that separating experimental and control erythrocytes from their respective sera in exactly the same manner may not affect them identically. A further complicating factor that may need to be taken in account in future work is suggested by the observation of Henriques & Ørskov (62) that after centrifuging erythrocytes



those at the top of the column may contain seventeen per cent more potassium than those at the bottom.

With these facts in mind, several puzzling results from the recent literature may be mentioned briefly. For example, Eisenman Hald & Peters (64) found that in human blood *in vitro*, on the addition of sodium chloride, potassium chloride or sodium sulphate there was no passage of base into the cells. In dehydrated human patients with serum-base deficiency, however, the administration of hypertonic salt solutions was followed by changes in the total base of the cells. A loss of potassium from human cells after hemorrhage has been reported by several workers in the past and again recently by Henriques & Ørskov (62). Hegnauer & Robinson (65) have found that after adrenalectomy in the cat, with the ensuing reduction of sodium and chloride and increase of potassium in the blood, there are corresponding changes in the erythrocytes, which they believe to have become "cation permeable." The objection that the erythrocytes analyzed during the control period may have been replaced during the course of the experiment by others having a different composition is met (66) by showing that in cats and rabbits which have received large intraperitoneal injections of isotonic glucose similar chemical changes of both the plasma and the cells occur too rapidly to be influenced by any appreciable formation of new cells.

These experiments, however, leave undecided the question whether the apparent increase in permeability to cations under the conditions in question is relatively permanent or merely transitory. The work of Davson, discussed above, suggests the importance of very temporary changes of the cells, which might conceivably be accentuated under the mechanical stress of passage through narrow capillaries in the circulation of the living animal. Further possibilities concerning the behavior of cells during centrifugalization, as well as possible differences in the composition of the mature cells contained in different blood reservoirs of the body are also worthy of consideration. Whatever the truth may ultimately prove to be concerning these points, the fact remains that on those who wish to postulate a simultaneous and long-continued permeability of the erythrocyte to both anions and cations rests the burden of explaining how the destruction of such a delicate cell by swelling under the influence of the Gibbs-Donnan equilibrium is avoided.

During the past two years a method applicable to man, and capable of throwing light upon the permeability to ions and other substances of the body cells collectively, has been used rather extensively. This consists in the introduction into the circulation of a diffusible substance, determining its concentration in the blood after a suitable period, and with proper deductions for losses in the urine calculating the volume of liquid through which it would have to be uniformly distributed to give the observed concentration. Two substances which on *a priori* grounds might be expected to distribute themselves throughout the entire water of the body seem actually to do so, namely urea and sulfanilamide (67). The same is at least roughly true of the excess urea formed after the introduction of ammonium chloride and of potassium introduced as potassium chloride (68, 69). This behavior of potassium is perhaps not surprising in view of the evidence summarized by Fenn (70, 71) that muscle cells on the whole seem to behave as if they were rather freely permeable to this ion.

On the other hand, sucrose, sulphate, thiocyanate, and chloride (68; 72, 73) seem to be distributed in a much smaller body of water, whose calculated volume is approximately the same for all, and which is generally believed to represent the extracellular water of the body—in other words, most of the body cells seem to be fairly impermeable to these ions. Recent work (74), indicating the ease with which the chloride of most tissues can be removed by a perfusion medium consisting of sulfates, is on the whole consistent with the view that in most of the tissues the chloride is extracellular, *i.e.*, that the cells are impermeable to it, though in some tissues the conditions seem to be rather more complex. Other experiments (75) on the distribution of chloride in frog's skeletal muscle after immersion in saline solutions also indicate an impermeability of the muscle cells to chloride.

Among anions, it should be noted that bicarbonate seems, in general, to move rather freely across cell boundaries, but this mobility is probably at least in part an indirect one, due to the peculiar properties of the carbon dioxide molecule. That the anions of the rapidly penetrating fatty acids also gain access to the interior of cells with considerable ease has been shown previously, and is confirmed by the recent work of Beck & Chambers (76). The manner of entrance in such cases is still uncertain, but these



authors are inclined to believe that an affinity of the ions themselves for lipids is involved.

The colored ions of certain dyes have frequently been employed in studies of permeability. Flexner & Stiehler (77, 78) have reported that at physiological pH basic dyes pass much more readily from the stroma to the epithelium of the mammalian chorioid plexus than in the reverse direction, while acid dyes behave in the opposite manner. Asphyxia tends to abolish these differences which the authors correlate with observed differences of 100 mv. in the oxidation-reduction potentials at the two sides of the membrane. These potential differences, the irreciprocal permeability of the chorioid plexus to dyes, and a chemical change in the character of the cerebrospinal fluid itself are all said to appear simultaneously about the forty-fifth day of development of the fetal pig. Rubinstein & Pevsner (79) also discuss the possible rôle of electrical factors in the one-sided permeability to dyes of frog skin. Meyer (80) has found that when the electric organ of the torpedo is stained with methylene blue and eosin alternate red and blue membranes appear in it, which he believes to be responsible for the development of electric potentials in the same manner as in model experiments of his own in which anion-permeable and cation-permeable artificial membranes were similarly combined.

*Factors modifying permeability.*—Several types of effects of oxygen on "permeability", using this term in its widest possible sense, have been reported by recent workers. At the one extreme are the results of Hunter (81, 82) who found no significant effect of oxygen absence on the permeability of the *Arbacia* egg to water and to ethylene glycol, or of mammalian erythrocytes to water, various non-electrolytes and ammonium salts. Collander & Holmström (83), on the other hand, have found that the uptake of acid dyes by the corolla cells of white tulips and hyacinths is greatly reduced in the absence of oxygen. A third type of result is reported by Runnström *et al.* (84) who found that sodium fluoride added to a yeast suspension in the absence of oxygen strongly inhibits subsequent respiration in the presence of oxygen, as well as anaërobic fermentation. The same substance added in the presence of oxygen and sugar, however, has no effect on respiration over a period of many hours; when added in the presence of oxygen but not of sugar a small effect is produced. The authors plausibly interpret

these results as being due to an increased permeability of the cells to sodium fluoride in the absence of oxygen, though certain other possible explanations have not been completely excluded.

In the more complicated cases of the active transport of materials against concentration gradients by methods which are as yet unknown, but which seem to involve an expenditure of energy, it is not surprising that the rôle of oxygen should be an important one. In the accumulation of electrolytes in the cell sap of *Valonia*, Steward (85, 86) has strongly emphasized the importance of respiration, as has Hoagland (13) in dealing with the same question for the roots of the higher plants. Lundegårdh (87) believes that there is a particularly intimate connection between respiration and the intake of anions by the roots of plants (*Anionenatmung*)—a view which Krogh (see page 14) suggests may perhaps be applicable to the intake of ions by fresh water organisms. Closely related to the effects of the absence of oxygen are those of the presence of potassium cyanide. Huf (25, 26) has found very striking effects of this substance on the passage of water and salts in the two directions across frog skin. Eckstein (88), however, reports that the irreciprocal passage of methylene blue is not affected by potassium cyanide. [See also in this connection Brooks (89).]

One of the more important general theories of narcosis is that narcotic substances cause a reversible change in the permeability of the cell, which is usually assumed to be a decrease. Effects, for the most part in this direction, have been found by Saubert (23) in studying the influence of a series of alcohols on the rates of exosmosis and endosmosis of water in cells of the plant *Chara*. They have in part been correlated with those obtained by the addition of the same alcohols to phosphatide coacervates (90). On the other hand, Bärlund (91) in an unusually careful piece of work on the same material, in which direct chemical analyses of the cell sap were made and permeability constants were determined, has found in the presence of 0.5 to 2.5 volume per cent of ether only reversible increases in permeability to ethylene glycol, hexamethylenetetramine, urea, and trimethyl citrate, and little change of permeability to lithium. Biskupski (92) using an electrical method with frog skin has reported with low concentrations of ethyl alcohol a reversible increase, with higher concentrations a reversible decrease, and with very high concentrations an irreversible increase in permeability. A recent paper by Curtis (93), how-

ever, suggests caution in drawing inferences concerning permeability from electrical changes alone.

Support for the "decreased-permeability" theory of narcosis has seemed to be furnished by the model experiments with collodion membranes reported several years ago by Anselmino. Ponder & Abels (94) have extended these experiments, and have confirmed the finding of Anselmino that various urethanes retard the diffusion of the thiocyanate ion across such membranes. However, they have obtained exactly the opposite effect with seven other anions, the rate of whose diffusion was increased in the presence of urethanes. They have also found that collodion membranes containing lecithin show an increased permeability to the thiocyanate ion, but not to the others studied, under the conditions in question.

The general result of these and of other recent studies of the influence of narcotics on permeability is to show that there is no single effect that can well be made the basis of a simple theory of narcosis. The complexity of the subject is illustrated by some recent observations by Jacobs & Parpart (95) on the erythrocytes of several species of mammals, which show that whereas the permeability to glycerol of the erythrocytes of one group of species is greatly decreased by the presence of low non-hemolytic concentrations of alcohols, the reverse effect is obtained with other species. Furthermore, with no species does the permeability to lipid-soluble substances and to thiourea seem to be decreased under these conditions, but that to the ammonium salts of mineral acids is decreased in all the species so far investigated. The mechanism of the latter effect is probably a decreased permeability to anions (54).

Many cases are known in which the "permeability" of a cell to a given substance changes markedly with the pH of the external medium. While in some cases the effect produced is on the cell itself, a much commoner condition is that in which it is the penetrating substance that is changed, as for example where weak acids or bases and their less readily penetrating salts are involved. An extremely interesting series of compounds for studying this phenomenon are the nitro- and halo-substituted phenols, which include compounds having almost any desired  $pK'$  value. Krahl & Clowes (96, 97) have performed a valuable service in determining  $pK'$  values ranging from 3.0 to 8.3 for 29 of these compounds. They have also studied the physiological effects of the same substances

on *Arbacia* eggs with different combinations of external and internal pH. Their general conclusion is that penetration of the cell occurs in the form of undissociated molecules, but that the resulting physiological effects are influenced in a somewhat complex manner by the internal equilibrium conditions. Tyler & Horowitz (98) using a smaller series of these compounds have likewise concluded that penetration occurs in the form of undissociated molecules, but their interpretation of the internal effects differs somewhat from that of Krahle & Clowes. Other papers in which a similar indirect influence of pH on permeability is shown are those by Albaum, Kaiser & Nestler (99) on 3-indole acetic acid, Martin & Field (100) on 2, 4-dinitrophenol, Ellisor & Richardson (101) on nicotine, Jacobs & Parpart (57) on ammonium salts, and Beck & Nichols (102) on fluorescent dyes.

As an example of a possible pH effect of an entirely different sort may be mentioned the work of Schulte (103) who found that root hairs of *Lepidium sativum* burst almost instantly in sufficiently acid solutions of different sorts. This effect can be progressively inhibited by increasing concentrations of sucrose and can be completely prevented at 0.1M. The author gives reasons for believing that it is one involving anomalous or negative osmosis, and he attempts to correlate it with the behavior of pollen tubes and other plant cells. Among other miscellaneous factors whose influence on cell permeability has recently been studied but which cannot be discussed because of lack of space are light (104), carbon dioxide (105),  $\beta$ -rays (106), constant electric currents (107), and the absence of electrolytes (108).

*Active transport and accumulation of ions.*—Since these complex phenomena seem to involve other factors in addition to permeability in its usual sense, no more than a very brief mention of certain recent work in this field will be attempted here.

A well-known instance of apparent ionic transport against a concentration gradient is that of chloride outward from the gills of marine teleost fishes, which has recently been reviewed by Keys (109). The opposite very interesting process of an inward transport of ions has been studied by Krogh (110, 111, 112) in the case of several fresh water animals. He has found that in the frog, after salt depletion by a fairly long exposure to distilled water, chloride can be absorbed from sodium chloride solutions more dilute than 0.01M. Bromide is also taken up in the same way, though with

somewhat more difficulty—which is not surprising in view of the similarity of the behavior of these two ions in the kidney of man and of the frog (113, 114). Forming an interesting contrast to chloride and bromide are iodide, nitrate and thiocyanate, which can be taken up at varying rates by simple diffusion, but not against concentration gradients. Furthermore, while chloride and bromide are not taken up by frogs having a normal salt content, nitrate is stated to penetrate such frogs at about the same rate as desalted frogs. When sodium is present, it is absorbed with chloride, but from solutions of ammonium chloride, calcium chloride, and potassium chloride, chloride is usually absorbed without being accompanied by a cation, though the skin has some powers of absorbing potassium. In such cases the penetrating anion is presumably exchanged for another anion, bicarbonate. The more ready passage of sodium than of potassium is in striking contrast to the behavior of these ions with most plant and animal cells and artificial membranes. In the gills of the goldfish the general permeability to water and to anions is lower than in the skin of the frog. In the crab, *Eriocheir*, the anion mechanism absorbs indiscriminately chloride, bromide, thiocyanate and cyanate but not iodide. No distinction is made by this animal between sodium and potassium. A process apparently similar to that just described has also been reported by Koch (115) and by Wigglesworth (116) for certain insect larvae.

The absorption of ions against concentration gradients in the mammalian intestine has been considered at some length in several recent papers (117, 118, 119, 120) which in addition to interesting experimental findings present a tentative theory to account for the latter. This theory postulates that in one region of the intestine sodium chloride passes inward with water at a constant rate, and that in another region water is returned through a membrane impermeable to salts at another constant but somewhat lower rate. Though a mathematical treatment of this hypothetical case gives a good formal agreement with the observed course of absorption, it should be noted that in the absence of filtration forces of far greater magnitude than any known to exist in the body a separation of water from the body fluids would involve osmotic difficulties as great as those associated with an inward passage of sodium chloride. It would also seem that such an explanation could scarcely be applicable to the apparently very similar and very

widespread behavior of the skin and gills of aquatic animals discussed above.

In plant physiology, accumulation of ions has been perhaps more carefully studied in the alga, *Valonia*, than in any other single object of either plant or animal origin. At present, however, while many of the main facts concerning this accumulation are entirely clear, there is still much difference of opinion as to their interpretation. One point of view, according to which a relatively simple mechanism that can be imitated in many of its aspects by a non-living model is involved, has been developed by Osterhout and his associates, and has been summarized in several recent reviews (9, 10) which may be consulted for full details. To these may be added a number of later papers (121, 122, 123, 124, 125).

A very different point of view, which rejects a simple physico-chemical explanation, and emphasizes especially the significance of complex metabolic processes in the process of accumulation is developed in a long paper by Steward & Martin (85), the many interesting details of which cannot be discussed here, but the gist of which can be obtained from a review by Collander (126). Light of a different nature has also been thrown on the behavior of *Valonia* by recent experiments by Brooks (127), who used radioactive potassium to follow in detail the manner of accumulation of this ion, and who emphasizes particularly the complications introduced by the attainment of high concentrations of potassium in the protoplasm before diffusion into the cell sap occurs.

The related subject of accumulation of ions by the roots of plants is dealt with in a review by Hoagland (13) whose general point of view is very similar to that of Steward & Martin mentioned above. Among the more recent papers in this field, one by Collander (128) in which the relative rates of accumulation of different ions by different species of plants under standardized conditions are compared, and that by Lundegårdh (87), already referred to, may be mentioned as containing material of particular interest to the student of cell permeability.

Though the factors involved in the movement of ions against concentration gradients in living organisms are still largely unknown and in most cases are probably highly complex, it is of interest to note that such movements are at least possible in certain simple models. That devised by Osterhout has already been mentioned. Teorell (129) has also recently considered the proper-

ties of another model of a somewhat different nature, in which by means of the driving force provided by the steady diffusion of a substance, perchloric acid, having anions and cations of very different mobilities, striking movements of other "passive" ions against their own concentration gradients could be produced. To what extent this and other models can be related to actual physiological processes remains for future work to determine.

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UNIVERSITY OF PENNSYLVANIA  
PHILADELPHIA, PENNSYLVANIA

## THE BIOLOGICAL EFFECTS OF RADIATION\*

BY JANET HOWELL CLARK

*School of Hygiene and Public Health, Johns Hopkins University  
Baltimore, Maryland*

Recent advances in radiation therapy hardly come within the scope of this review, but Luce-Clausen's article on the clinical aspects of ultraviolet therapy (1) sums up admirably the current work along this line, and recent advances in the therapeutic use of x-rays and radium are summarized in reports of the Medical Research Council, in the *Year Book of Radiology*, and in other publications. This review will deal with current investigation on the biological effects of radiation, with emphasis on fields in which there has been particular interest during the last two or three years. An effort has been made to limit the discussion to work published between January 1936 and July 1938 although some reference is made to earlier papers.

### LETHAL EFFECT OF RADIATION ON CELLS

*Ultraviolet radiation.*—The discovery of the lethal action of radiation on bacteria in 1877 first called attention to the importance of radiation in biology. Excellent quantitative studies on this effect were made as early as 1928 but new papers are steadily being published. These recent studies are largely undertaken with the purpose of comparing the lethal effect of measured radiation on different organisms and virus proteins or of finding a new clue to the mechanism of injury.

Duggar & Hollaender (2) compared the lethal action of ultraviolet radiation on tobacco mosaic virus and the cells of *S. marcescens* and found the resistance ratio of virus to bacteria about 200:1. Vegetative stages (*B. subtilis* and *S. marcescens*) were found to be more sensitive to radiation than the spores but the survival curves were generally comparable. A similar result was found by Hercik (3) with *B. megatherium*. Twice as much energy was needed to kill the spores as the vegetative forms. In a later paper (4) Hollaender & Duggar found tobacco mosaic virus forty times as resistant as *Escherichia coli* at  $\lambda$  2,650 Å. The sensitivity of bacteria was found to be at a maximum at  $\lambda$  2,650 Å whereas the

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virus showed increasing sensitivity with decreasing wave length down to  $\lambda$  2,250 Å. Hollaender & Claus (5) found differences in the energy needed to inactivate young, old and standard cultures. Dreyer & Campbell-Renton (6) found that the relative bactericidal effect of different regions of the spectrum varied with the organism exposed and in a later paper Campbell-Renton (7) reported some strains of bacteriophage to be 6 to 8 times as sensitive as others to the lethal effect of ultraviolet radiation. Inactivation of poliomyelitis virus (8, 9) and rabies virus (10) has been reported and it is stated that in the case of rabies virus the virulence may be destroyed by exposure to ultraviolet radiation without complete loss of its immunizing power.

Experiments on the differential susceptibility of a number of protozoans to ultraviolet radiation (11) have brought out the interesting point that there is no correlation between size or shape of the organisms and their resistance to radiation. Another paper (12) reports a change in resistance of paramoecia to radiation with the age of the culture.

Wells (13, 14, 15) has presented evidence to show that air-borne infection may be due to slowly settling dried residues of infected droplets and has proved that influenza virus can be recovered after being suspended in the air for at least thirty minutes. These suspended virus droplets are inactivated by exposure to ultraviolet radiation. The lethal effect of a given dose of radiation was found to be greater at a low humidity.

Sharp (16), in a quantitative study of the effect of nearly monochromatic radiation (2,537 Å) on bacteria, atomized into air and passed through a tube where exposure to radiation takes place, found 26,200 ergs per sq. cm. necessary to kill all organisms.

These experiments by Wells have led to the use of ultraviolet radiation of intensity sufficient to kill air-borne organisms, in operating rooms as a method of combating infection (17, 18, 19, 20). While it is doubtless possible to sterilize the air in this way the injurious action of ultraviolet radiation on tissues should be borne in mind and care taken to prevent possible injury through prolonged exposure of open wounds.

*X-rays and radium.*—Recent work on the lethal effect of x-rays and  $\gamma$ -rays on cells has been summarized in reviews by Scott (21) and Crowther (22). The chief point emphasized in these discussions is the fact that different types of living cells and organisms

vary greatly in their radio-sensitivity. Protozoa are the most radio-resistant tissues and the eggs of insects the most radio-sensitive, there being a ten thousand fold difference in radio-sensitivity between these two extremes. The problems being investigated can be classified under three heads: (a) the relationship between the dose of x-rays, or  $\gamma$ -rays, and the proportion of cells killed, (b) the influence of mitosis on radio-sensitivity, and (c) the changes in radio-sensitivity of cells due to embryological development or change in environment.

Curves in which the dose of radiation is plotted against the percentage of survivors have been obtained for various cells and organisms by a number of different observers. The shape of the survival curve differs, being sigmoidal in some cases and exponential in others. The curves may be explained theoretically as the consequence of the distribution of variation of the resistance of individual cells to the radiation, or products of radiation, this being called the individual variation theory or the poison hypothesis. Or they may be accounted for on the basis of the relation of the dose to the percentage of cells receiving a sufficient number of quantum hits to produce death (quantum hit theory).

Scott (21) in the second part of his report gives the results of his own experiments which were performed to test the quantum hit theory. A more or less uniform population of eggs of *Calliphora erythrocephala* were subjected to injuries produced by a number of different agents. He found that the distribution of injury produced by x-rays is similar to that produced by cold, asphyxia and carbon dioxide. These results are not compatible with the view that the distribution of injury can be explained in terms of the quantum hit theory. Crowther (22) on the other hand maintains that, while variation in radio-sensitivity of the population would account for a sigmoid survival curve, the exponential curve, which is often reported, can only be explained in terms of the target hypothesis. Lea, Haines & Coulson (23) also present evidence against the poison hypothesis and in favor of the target hypothesis. They exposed bacteria in thin gelatin films to  $\alpha$ - and  $\beta$ -rays and, in a later study, exposed *B. coli* in aqueous suspension to  $\gamma$ -rays, finding exponential survival curves in every case.

**Neutrons.**—Recent advances in nuclear physics have focussed the interest of many investigators on the biological effects produced by neutrons. The neutron is a particle of mass 1 and charge

0. It is believed to be one of the two fundamental building stones from which all nuclei are constructed, the other being the proton (mass 1, charge +1). Neutrons may be ejected from various nuclei by bombarding them with protons, deuterons,  $\alpha$ -particles or  $\gamma$ -rays. In most of the experiments reported so far neutrons are produced by bombarding a target of light metal, usually beryllium, with deuterons, the nuclei of heavy hydrogen, which have been accelerated to very high speeds in a cyclotron. In most of the experiments quote a beam of five million volt deuterons, emerging from the cyclotron, impinge on the beryllium target and produce a beam of neutrons.

Neutrons, like x-rays,  $\gamma$ -rays,  $\beta$ -rays and  $\alpha$ -rays produce biological effects because of the ionization they cause. X-rays and  $\gamma$ -rays, impinging on matter, produce electrons which cause a small degree of ionization over a long path. Neutrons, on collision, give their energy to protons (hydrogen nuclei) or to other light nuclei and these produce intense ionization over a short path. Carbon nuclei give more intense ionization over a shorter path than protons. As biological effects on individual cells depend more on ionization density than on total number of ions it is reasonable to expect neutrons to be more effective biologically than x-rays and  $\gamma$ -rays of the same intensity as measured by total ionization, and such is found to be the case. In contrast to x-rays, neutrons can penetrate great thicknesses of dense substances such as lead and are more readily absorbed by materials rich in hydrogen.

These theoretical deductions have been verified experimentally for Zirkle, Aebersold & Dempster (24), using as test objects the inhibition of hatching of *Drosophila* eggs, the retardation of growth in the roots of wheat seedlings, and the injurious effect on fern spores, found that neutrons are two to five times more injurious than x-rays, the ratio varying with the biological object used. In a later paper (25) a neutron to x-ray ratio of effectiveness of two to ten is reported.

Zirkle & Aebersold (26) have also shown that neutrons are more effective than x-rays on Sarcoma 180 in vitro, and Lawrence (27) found the same result with mammary carcinoma.

Lawrence & Lawrence (28) and Lawrence & Tennant (29) radiated mice with neutrons produced by five million volt deuterons and also with 200 kv. x-rays. The clinical, bacteriological and histological pictures were the same in both cases, death being ap-

parently caused by tissue destruction. Lymphoid tissue, bone marrow, and the mucosa of the small intestine are especially radio-sensitive. The lethal dose was found to be smaller in the case of neutrons.

Table I, quoted from Lawrence (30), gives the neutron to x-ray ratio of effectiveness for a number of biological test objects. The intensity of the x-rays is measured in roentgens (r); the r of neutrons was the dose which produced total ionization equal to that produced by a roentgen of x-rays.

TABLE I  
COMPARATIVE EFFECTS OF X-RAYS AND NEUTRONS

Object	X-ray	Neutron	Ratio
Mammary carcinoma	3,600 r	700 r	5.1
Mice (lethal effect)	800 r	200 r	4.0
Drosophila eggs	180 r	87 r	2.1
Wheat seedlings	600 r	120 r	5.0
Fern spores	52,000 r	21,000 r	2.5

A very illuminating discussion of the relative effects of various forms of ionizing radiations is given by Zirkle (31). In Table II is given material from Table I and Figure 2 of Zirkle's paper showing the specific ionization (ions per micron of water depth) and the ionic effectiveness (biological effectiveness per ion), as determined by the effect on fern spores.

TABLE II

Particle	Mass	Charge	Specific ionization, ions per $\mu$	Ionic effectiveness
Electron	1/1800	-1	10- 150	0.12-0.13
Proton (H nucleus)	1	+1	500-1,900	0.13-0.20
$\alpha$ -particle (He nucleus)	4	+2	3,000-7,500	0.25-1.0
Carbon nucleus	12	+6	{ Greater than 7,500	
Nitrogen nucleus	14	+7		
Oxygen nucleus	16	+8		

X-rays and  $\gamma$ -rays produce electrons and, in the region of electron specific ionization, the ionic effectiveness is practically constant. Therefore the biological effects of x-rays and  $\gamma$ -rays should be practically independent of wave length. This is in

general the result found experimentally. Neutrons ionize largely by accelerating recoil protons, although there may be some effect from accelerating nuclei of carbon, nitrogen and oxygen atoms so that the ionic effectiveness may vary with the constitution of the tissue.

When the ionic effectiveness of the neutron is twice that of x-rays the ionization must be due largely to protons. When it is five times as great some of the ionization must be due to other light nuclei. In general it can be concluded that the greater the specific ionization of a radiation the greater will be its ionic effectiveness and the greater the injury for a given amount of energy absorbed. Also the rate at which ionic effectiveness increases with specific ionization will vary with the type of cell radiated.

#### INJURIOUS EFFECTS FROM SUB-LETHAL EXPOSURE

The primary effect produced by radiation is ionization but this is followed by secondary effects. Ultraviolet radiation, x-rays and radium rays all injure the mechanism that controls the growth of the cell and, if exposure is sublethal, secondary changes are produced, which may affect the cell in many ways. Henshaw (32) has shown that the effect on nuclear material is responsible for a slowing in cell division after exposure to x-rays but that all parts of a cell are sensitive to radiation. Not only cells but most organic substances are definitely, and often irreversibly, altered by exposure to radiation.

Injuries produced by ultraviolet radiation have been summarized in a recent review (33). In higher animals ultraviolet radiation is absorbed entirely in the skin and eyes and produces its effects in these tissues. The denaturing effect of ultraviolet radiation on lens proteins, with its possible relation to senile cataract [Clark (34)], and the injurious action of radiation on skin tissue, leading in extreme cases to the production of skin cancer, are two of the problems that have attracted the attention of investigators recently.

*Tumors from radiation.*—The acceleration of the appearance of tar cancer in radiated mice, and the production of skin cancer by exposure to ultraviolet radiation, were noted by Findlay (35) in 1928. This effect has been studied extensively during the last few years by Roffo (36, 37). Cancers were produced in white mice and rats by daily exposure to a quartz mercury arc or to sunlight.



The incidence of carcinoma and sarcoma was approximately equal. These results have been confirmed by Beard (38). He exposed rats to ultraviolet radiation daily for one year and produced sarcoma or carcinoma of the eyes, ears, and heads in forty per cent of the experimental animals. It is interesting also to note that cataracts appeared after about two months irradiation.

Roffo linked the development of tumors with an increase in the cholesterol content of the skin after radiation as he found a large concentration of cholesterol in tumors and a higher concentration in radiated skin than in normal skin (39, 40).

More recently Knudson and his associates (41) have confirmed the results of Roffo and Beard by producing tumors in the skin of radiated animals and by finding a high cholesterol content in tumors as well as in radiated skin. These results indicate need of caution in the use of sunbaths but Miescher in a discussion of the subject (42) gives it as his opinion that exposure to the sun is harmless if sharp reactions are avoided.

Continuous weak  $\gamma$ -radiation has also been shown to produce cancer. Ross (43) placed platinum tubes containing 0.1 mg. of radium in the tissues of rabbits. Six out of nine animals developed malignant growths in the neighborhood of the tube. Some were carcinoma and some sarcoma. She concluded that weak  $\gamma$ -radiation is carcinogenic, that epithelial and connective tissues are equally affected, and that the type of growth depends on the nature of the tissue in which the tube is embedded.

Since Findlay's observation that ultraviolet radiation will accelerate the formation of tar cancer in mice a number of observers have shown that x-rays and radium rays also will favor the action of a number of carcinogenic agents. Mayneord & Parsons (44) found that exposure of mice to x-rays increased the number of sarcomas induced by a carcinogenic substance. Motttram (45) found that, in mice painted with benzopyrene twice a week for ten weeks, and exposed to doses of  $\gamma$ -radiation insufficient to destroy the epidermis (176 to 1,584 r), the incidence of epithelial tumors was greater than in non-irradiated animals. In a later study Mottram (46) painted mice twice a week for ten weeks with benzopyrene and on the sixtieth day gave them a single exposure of  $\beta$ -radiation with doses varying from 800 to 2,500 r. This treatment produced both benign and malignant tumors. This finding

emphasizes the danger of exposing precancerous lesions to radiation.

Burrows and his coworkers (47) found that sarcomas could be produced by exposing inflammatory lesions, caused by the injection of kaolin and finely powdered silica in olive oil, to a single dose of x-rays (600 r). They suppose that the inflammation causes a concentration of carcinogenic agents in the tissue.

Burrows & Mayneord (48) found that, when cholesterol previously exposed to x-rays was injected into mice, tumors appeared in two of the twenty treated animals. It would seem that this experiment might have shown more striking results if the cholesterol had been exposed to ultraviolet radiation rather than to x-rays. Roffo reported activation of cholesterol on exposure to ultraviolet radiation and, although Mayneord (49) found no ultraviolet phosphorescence from radiated cholesterol, as claimed by Roffo, he did find that it emits an active vapor.

Doniach & Mottram (50) find that benzopyrene, tar and dibenzanthracene all sensitize the skin of animals to visible light. Mice after being painted 2 times a week for 3 weeks become red and edematous on exposure to sunlight. Radiation therefore may have a double action if, on the one hand it sensitizes the skin to visible as well as ultraviolet radiation, and on the other hand concentrates in the skin substances such as cholesterol which may possibly become carcinogenic on exposure to ultraviolet rays, x-rays or radium. Further reference will be made to this point in another section of this review and it is obvious that it is a field that suggests many fruitful lines of research.

Mutations.—X-rays and  $\gamma$ -rays of sublethal intensity produce an increase in the rate at which genetic mutations occur. Scott's report (21) gives a very complete summary of experiments along this line. The genetic effect of x-rays and  $\gamma$ -rays is found to be (51) independent of the wave length, of the temperature during radiation, and of the time interval between doses. It is proportional to the dose of radiation. The genetic action of radiations is to alter the arrangement of the genetic material of a cell rather than to produce an injury. It is exerted on the structures of the cell which control growth and is independent of the cell's metabolic processes [Scott (21)].

Mitosis.—An effect which is more clearly an injury is the delay in mitosis that is observed after sublethal exposures to radiation.

A recent paper by Giese (52) states that retardation of cleavage of sea urchin eggs was proportional to the dose of ultraviolet radiation ( $\lambda$  2,537 Å) and that no stimulating effects were found.

All investigators agree that cells are most sensitive to injury by x-rays and radium at one or other phase in mitosis but Packard (53) has pointed out that the variation in radio-sensitivity during the mitotic cycle is small compared with the variation in sensitivity of different types of cell. Lea (54), in a discussion of recovery from sublethal doses of radiation, brings out the important point that if cells were injured to the same extent in all phases of the mitotic cycle, and were capable of some recovery with time, those radiated at or just before mitosis would show the effect of delayed division whereas those radiated in the resting phase would recover sufficiently to divide at the normal time and would consequently seem to have been less affected.

#### STIMULATING EFFECTS FROM SUB-LETHAL EXPOSURE

Packard is of the opinion that x-rays and  $\gamma$ -rays do not directly stimulate the normal activities of the cell. The primary effect is always an injury from which the cell may recover perfectly, the acceleration of some normal cell processes which has been observed being a temporary response to injury. It is however possible that the products of cell injury, whether ultraviolet rays or x-rays are used, may contain growth promoting substances which have the power of stimulating uninjured cells.

*Growth promoting substances.*—Sperti, Loofbourow and their associates (55, 56, 57) report that yeast cells injured by ultraviolet radiation produce a substance, or substances, which promote growth when added to non-irradiated suspensions of yeast or to cultures of embryonic chicken heart. The centrifuged cell-free fluid from dead irradiated cells was also found to be beneficial in wound healing. The nature of the growth promoting substance, or substances, has not been determined and it is not known if similar substances are produced by cells injured by agents other than radiation.

*Mitogenetic Radiation.*—The stimulating effect claimed for mitogenetic radiation falls in a different category. Here ultraviolet radiation, so feeble as to be incapable of measurement by physical means, is claimed to increase the rate of cell division. These rays are said to be in the ultraviolet region of wave length 1,900 Å to

2,500 Å, but Bateman (58) in his review severely criticizes this conclusion. This radiation, said to be emitted by cells in certain stages of development and by certain chemical reactions, is called "mitogenetic" because of its stimulating influence on mitosis. This radiation was first reported by Gurwitsch in 1922. His results are accepted by many observers but the more critical investigators question their authenticity and ascribe the positive results obtained to faulty technique.

A number of comprehensive reviews [Bateman (58), Hollaender (59, 60)] have summarized the results critically up to 1935. There are two methods of detection, biological and physical. The most satisfactory biological methods of detection are the stimulation of mitosis in onion roots, budding of yeast and growth of bacteria. Apparently even investigators reporting positive results have "periodic" failures and the best controlled work has been entirely negative. Kreuchen & Bateman (61), using yeast as detector with a number of inductors, obtained negative results; those of a recent thorough and well controlled study in which bacteria (*E. coli*) were used as detector [Hollaender & Claus (62)] was also negative. Bateman quotes early work by Moissejewa in which it is shown that the slightest pressure in the handling of the onion root detector will cause asymmetry of mitosis so that many effects reported may be due merely to manipulation of the material. It seems justifiable to conclude that the variability of biological material makes biological detectors unsatisfactory and untrustworthy.

If mitogenetic radiation exists it should be capable of detection by physical methods. Rajewsky in 1931 reported positive results with a modified Geiger-Müller counter sensitive to 14 to 140 quanta per sq. cm. per sec. His results were confirmed by Frank and Rodionow in 1932 with data implying an intensity of mitogenetic radiation of about 600 to 2,000 quanta per sq. cm. per sec. (See Bateman's review.) But Gray & Ouellet (63) with a Geiger-Müller counter sensitive to 50 quanta per sq. cm. per sec. found no indication of radiation from fertilized sea urchin eggs or from growing yeast, while Lorenz (64) failed to detect mitogenetic radiation from onion root, mouse embryo and mouse sarcoma used as inductors with a Geiger-Müller counter sensitive to 10 to 15 quanta per sq. cm. per sec. Hollaender (62) obtained negative results with a less sensitive form of counter (500 quanta per sq. cm. per sec.) which was so sensitive to the disturbing influences of humidity

and electrical potentials that great care had to be taken in bringing moist or ungrounded bodies near the photon counter. He attributes the positive results that have been reported to errors due to these disturbing influences.

It seems therefore increasingly evident that mitogenetic radiation is non-existent. If it occurs at all, it must have an intensity less than 50 quanta per sq. cm. per sec. One might expect some chemiluminescence to accompany growth activity but, as Bate-man points out, the quantum energy of the radiation accompanying a chemical reaction cannot exceed the heat of reaction so that chemiluminescence would be more likely to fall in the infrared and visible than in the ultraviolet region. Instances of ultraviolet chemiluminescence in liquid systems are rare and physical considerations lead one to conclude that the phenomenon, though possible, is rather improbable.

If the phenomenon does exist it is hardly conceivable that radiation of such exceedingly low intensity would have any significant effect. Stimulating effects produced by exposure to very small doses of ultraviolet radiation have often been reported but it has never been clear that the results indicate a real stimulation. Hollaender recently (62, 65) found an "initial increase" and an "extended lag phase" in cultures of *Escherichia coli* exposed to monochromatic ultraviolet radiation (2,500 Å) but only at intensities very much higher than those claimed for mitogenetic radiation (50,000 quanta per sq. cm. per sec.). At the energy level of the supposed mitogenetic rays no stimulation was produced.

These recent studies seem therefore to have abundantly proved, first, by physical methods, that if growing cells give out mitogenetic radiation it has an intensity less than 50 quanta per sq. cm. per sec., (since it cannot be detected by the photon counter), and, secondly, that if ultraviolet radiation of this intensity were emitted it would not produce a stimulating effect on living cells. The evidence in favor of the existence of these rays rests entirely on experiments with biological detectors which give variable results and where the sources of error are greater than the effects obtained. The existence of mitogenetic radiation would therefore seem to be amply disproved. Investigators who have followed the enormous literature that has accumulated in this field can be grateful to the exhaustive and careful work that has dispelled this particular illusion.

## RECOVERY FROM RADIATION EFFECTS

When inert materials such as protein solutions are radiated the denaturation produced by radiation appears to be irreversible. However in living cells processes of growth and repair may bring about complete recovery from the effects of radiation provided the injury is not too severe. There is however little experimental work to report along this line.

A very careful study of the rate of recovery of skin from exposure to x-rays and  $\gamma$ -rays has been made by Quimby & MacComb (66, 67). They find by studying the effect of successive doses that 45 per cent of the initial dose remains effective the second day.

Shreiber (68) studied the recovery of yeast cultures from the effect of ultraviolet radiation and concluded that repair of injury is stimulated by subsequent exposure to infrared radiation.

## THE TEMPERATURE COEFFICIENT OF RADIATION EFFECTS

*Ultraviolet radiation.*—Experiments by Clark on protein solutions (69, 34) have shown that the familiar process of denaturation and coagulation produced by exposure to ultraviolet radiation takes place in three steps. The first step, the light denaturation of the protein molecule, is a physical change which is independent of temperature and hydrogen ion concentration. The second step is a chemical reaction between the light denatured molecule and water with the high temperature coefficient of 10. The third step is the flocculation of light and heat denatured molecules to form a visible coagulum. This is a physical change which occurs only at the isoelectric point in salt-free solution and near the isoelectric point when salts are present.

Lethal and sublethal changes brought about in cells by radiation, if related to changes in cell proteins, may be initiated by the first step only and if so would be independent of temperature. If, however, these changes are the result of protein coagulation a high temperature coefficient would be expected. The temperature coefficient of the biological effects of radiation seems therefore a fruitful field for investigation in looking for a clue to the mechanism of these effects.

Preliminary observations have shown that the lethal effect on paramoecia is probably independent of temperature (70). The temperature coefficient of production of erythema in human skin

was found to be 2.3 [Clark (71)]. Merker (72) determined the lethal dose of ultraviolet radiation for flat worms at temperatures ranging from 0° to 30°C. and his results indicate a temperature coefficient of 1.5 to 2.2.

*X-rays and  $\gamma$ -rays.*—The influence of temperature on the effects produced by x-rays and  $\gamma$ -rays has long been a subject of controversy. The majority of investigators have looked for an effect of temperature during exposure. This would not be expected as the primary effect of radiation has always been found to be independent of temperature. The negative results reported and summarized in Scott's report (21) are therefore to be expected. It is the secondary changes following radiation that might be expected to have a temperature coefficient which would be high if protein coagulation were involved but might be lower if the effects were related to other organic constituents of the cell. If radiation is long continued the temperature applied during radiation may have some effect on the secondary processes. This would account for the positive results of Dognon & Packard (see Scott for references).

Scott also gives a table summarizing the influence of the temperature at which a tissue is incubated subsequent to radiation. Owing to the nature of the tissues used the temperature has seldom been raised above 25°C. in these experiments. One would not expect the effect of temperature on secondary reactions to be marked until temperatures between 35° and 40°C. are reached. The only paper quoted by Scott [Strangeways & Fell (73)] in which the temperature after radiation was sufficiently high to lead one to expect a definite effect gave positive results. In their experiments they exposed 6 day chick embryos to x-rays *in ovo*. After a given dose followed by incubation at 38°C. mitosis was almost or completely absent and tissue taken from embryos radiated and incubated for 24 hours, showed no trace of growth when explanted *in vitro*. If, after the same dose, the embryos were kept at 5°C. for 24 hours, degenerative changes were delayed, if not arrested, and tissue fragments showed fair growth with mitosis when cultivated *in vitro*.

Owing to the latent period of the changes produced by x-rays and radium rays it seems probable that the primary change must be followed by a secondary change with a definite temperature coefficient before marked injury occurs. If the second change is



prevented or delayed, by lowering the temperature after radiation, injury should be delayed and a certain amount of recovery may take place, as was apparently the case in Strangeways' experiments, unless the process of recovery has a higher temperature coefficient than the process of injury. If the secondary changes are accelerated by raising the temperature after radiation the injury should be more rapid and extensive, as little repair could take place.

It was reported some years ago that heat applied to the skin after exposure to x-rays will increase the degree of erythema. This observation has been recently confirmed [Meyer & Mutscheller (74)]. Warren (75) found that fever treatment producing a rectal temperature of 41.5°C. increased the lethal effect of x-rays on Brown-Pearce rabbit epithelioma *in vivo* and gives it as his impression that fever therapy combined with fractional x-ray therapy would enhance the effectiveness of the x-rays. Similar results were reported by Meyer & Mutscheller (74).

A clearer conception of the relation of temperature to primary and secondary radiation effects should lead to advances of both clinical and theoretical interest.

#### UNDERLYING MECHANISM OF RADIATION EFFECTS

The end results observed after radiation, such as lethal action and delayed cell division, must of course be initiated by changes in the organic substances of the cell and their molecular organization or by changes in cell physiology or cell structure. Investigations on these fundamental aspects of radiation biology are in progress along a number of different lines.

*Cell metabolism.*—Although it has been shown that injury from radiation may lead to metabolic changes in cells these changes seem to be the result, not the cause, of the injury to growth.

Packard (53) stated in 1933 that the primary biologic effects of x-rays are changes in the hydrogen-ion concentration of protoplasm, in the permeability of the cell membrane, in viscosity and in the respiratory rate. Since that time many papers have been published on the oxygen consumption of tissues. These results are reviewed by Scott (21) and the one definite conclusion reached is that when a tissue is radiated its growth is inhibited before its respiratory mechanism is affected, which fact indicates that the primary action is not exerted on the respiratory mechanism.



Crabtree (76), it is true, found that the oxygen consumption rate of normal and tumor tissue fell after exposure to  $\beta$ - and  $\gamma$ -rays. Mottram (77) confirmed these results with bean roots. Bancroft & Kinsey (78) report that x-rays lower the R. Q. of rat sarcoma both *in vitro* and *in vivo*. On the other hand Boell, Ray & Bodine (79) conclude from experiments on grasshopper eggs, that x-rays (2,040 to 5,000 r) do not primarily affect the respiration mechanism of the embryonic cell but rather bring about fundamental changes in protoplasmic organization. And an extensive investigation of the relationship between the action of x-rays and metabolic processes has been carried out by Eichholz and his associates and reviewed by Reis & Kluge (80) which supports the conclusion that the respiratory system of the cell plays no significant part in the process by which x-rays and  $\gamma$ -rays injure living cells.

Conflicting results have been reported on the effect of ultraviolet radiation on oxygen consumption but none of the work is of very recent date.

The nitrogen metabolism of the cell has been found to be unrelated to the mechanism by means of which x-rays and  $\gamma$ -rays inhibit cell division (81).

*Hydrogen-ion concentration.*—Szendro and his associates (81) have shown that the isoelectric point of the tissue proteins is unaltered by radiation. It has been suggested that modification of the hydrogen-ion concentration of the cell by environmental factors may change the sensitivity of the cell to radiation. Scott (21) in original observations given in the second part of his report found, using the eggs of *Calliphora erythrocephala* as test object, that the action of x-rays is independent of the hydrogen-ion concentration inside the cell, when this is increased by carbon dioxide.

Zirkle (82, 83), on the other hand, found that ammonia inhibits the action of x-rays on fern-spore germination and Marshak (84) reports that ammonia decreases the sensitivity of chromosomes to x-rays. These observations are related, by the authors, to a change in the charge of the protein constituents of the cell with pH.

Clark (69) has shown that the initial change brought about by ultraviolet radiation in proteins is independent of hydrogen-ion concentration, but that some secondary changes are in-

fluenced by it. It seems probable therefore that some of the end results of radiation may be affected by the pH of the cells.

*Proteins.*—The changes produced in proteins by ultraviolet radiation have been studied by many investigators and the results have been reviewed by Clark (85), Arnow (86) and others. The denaturation of the protein molecule on exposure to radiation has long been considered to be a fundamental effect which may lie at the bottom of many more complex radiation changes. The changes produced in proteins themselves are complex, the initial light denaturation being followed by a secondary heat change before visible coagulation of the protein takes place (69). The question that is yet unsolved is the nature of the initial light denaturation. New work on the size and structure of large organic molecules has shown that the individual properties of large protein molecules depend on molecular orientation and may be profoundly affected by slight structural changes. Attempts are being made to find out what rearrangement of the protein molecule takes place on exposure to radiation.

Allen and his coworkers (87) have shown that ammonia is liberated from a number of amino acid compounds on exposure to ultraviolet radiation or to cathode rays.

Wels (88) has for some time emphasized the fact that sulphydryl groups, that are not present in the native state, appear when the protein is denatured by ultraviolet radiation. Mirsky & Anson (89) have also noted that coagulation of proteins, when produced by heat, light and acids is accompanied by changes in their sulphydryl and disulfide groups which indicates a change in the internal configuration of the proteins. The presence of sulphydryl groups after exposure to radiation would promote oxidations and reductions in radiated tissues. As the sulphydryl groups appear on coagulation they may be associated with the secondary change rather than with the primary change produced by radiation.

*Surface films.*—Another method of attack on the behavior and structure of organic molecules is the study of surface films. This is particularly important in considering the structure and behavior of cell membranes. Mitchell (90, 91, 92) has made a study of the role of molecular orientation in photochemical reactions in monolayers. He radiated monolayers and observed the consequent reactions by measurements of phase-boundary potential and surface

pressure. This is a method of studying the action of radiation on substances of physiological importance under conditions comparable with those occurring naturally. Egg albumin films, on exposure to ultraviolet radiation, show an increase in phase-boundary potential of 20 to 40 millivolts, which is followed by a liquefaction of the solid film. He believes the process to be a photochemical hydrolysis of the CONH linkages of the main polypeptide chains and thinks that only those CONH groups that are adjacent to the aromatic side chains are affected. He also exposed films of stearic anilide on hydrochloric acid substrate. On exposure to ultraviolet radiation, of wave length less than 2,500 Å, the phase-boundary potential increased 200 to 250 millivolts and the films liquefied and contracted. The end product is stearic acid. Probably the quantum is absorbed in the region of the benzene nucleus and this is followed by rearrangement of the molecule and breaking of the chain at the CONH linkage because of the low energy of activation required for its hydrolysis. These experiments form a new and valuable form of attack on the problem of the fundamental mechanism involved in photochemical changes.

*Lipoids and related substances.*—Lipoids are an important constituent of the cell, studies of cell membranes indicating that they are formed from a mixture of proteins and lipoids. As radiation injures the cell membrane a study of the photochemical changes produced in lipoids as well as in proteins is important.

Some years ago the discovery of the isomeric changes produced in ergosterol by ultraviolet radiation explained the therapeutic effect of ultraviolet radiation in rickets. Now the production of cancer by continued exposure of skin to radiation and the close structural similarity between carcinogenic hydrocarbons and sterols has suggested to a number of investigators that carcinogens may be synthesized in the tissues by radiation (1). Cook (93) suggested some years ago that carcinogenic hydrocarbons may be formed in the body from sterols and bile acids by abnormal metabolic processes. The concentration of cholesterol found in radiated skin (39, 40, 41) and the fact that cholesterol (49) and skin lipoids (94) become activated on exposure to radiation leads to the suggestion by Luce-Clausen (1) in her recent review that the formation of carcinogens from sterols may be effected by radiation.

A survey of the field of radiation research now occupying the

attention of investigators indicates that the action of radiation on sterols, with its possible relation to the synthesis of carcinogenic hydrocarbons in the skin, would seem to be a particularly interesting field for further investigation.

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SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY  
BALTIMORE, MARYLAND

## PHYSIOLOGICAL ASPECTS OF GENETICS\*

BY G. W. BEADLE

*School of Biological Sciences, Stanford University, California*

While recognizing that all of genetics must have a physiological basis—and ultimately a physico-chemical one—geneticists for many years have been concerned largely with the numerical and geometrical aspects of heredity. Within recent years, however there has been an evident and growing interest in problems that have to do with the relation of hereditary mechanisms and hereditary units to other branches of biology. A surprisingly large number of specific physiological reactions are now known to be under the control of genes. As an example, Trimble & Keeler have verified and extended earlier observations on differences in nitrogen metabolism in dogs. Most breeds of dogs excrete only a small amount of uric acid (0.2 to 0.4 per cent of the total urinary nitrogen). Individuals of the Dalmatian breed, however, excrete less nitrogen as allantoin and more as uric acid (2 to 3 per cent of the total nitrogen). Trimble & Keeler have verified the earlier suggestion of Onslow that high uric acid excretion is differentiated from low uric acid excretion by a single recessive gene. The geneticist is, in fact, confident that all processes characteristic of living organisms are ultimately gene-controlled.

Obviously, in any review of those aspects of current genetic research that may be of particular interest to physiologists, arbitrary selection must be made if such a review is to be confined within reasonable limits. Except for a brief consideration of work on protozoa, the entire subject of sex determination and differentiation has been omitted. Similarly, considerations of the problem of the role of cytoplasm in heredity and of the subject of mutation, have been greatly curtailed. Even with these and other omissions, little more than reference can be made to many papers.

For general reviews of physiological genetics see Henke (1, 2) and Goldschmidt (5). Gulick (1, 2) has reviewed information bearing on the nature and properties of the genes.

\* Received October 6, 1938. This review is largely limited to work published during 1937 and early 1938. No attempt has been made to cite all the literature, even on those particular aspects of the subject chosen for review.



## GENETIC BASIS OF FLOWER COLORS

During recent years the work of Onslow and others on the chemical basis of genetically differentiated flower colors has been continued by Scott-Moncrieff (1, 2, 3) and by other workers. Scott-Moncrieff has shown that many simple chemical differences in anthocyanins and related pigments are controlled by single gene differences, as illustrated by the following list, condensed from Scott-Moncrieff (1):

<i>Process involved</i>	<i>Plant</i>	<i>Dominant</i>	<i>Recessive</i>
Yellow plastid production (carotenoid pigments)	<i>Cheiranthus cheiri</i>	Yellow	White
Yellow anthoxanthin production	<i>Antirrhinum majus</i>	Yellow	White
Anthoxanthin copigment production	<i>Primula sinensis</i>	Magenta	Red
General anthocyanin production	<i>Dahlia variabilis</i>	Scarlet	Yellow
Specific anthocyanin production (pelargonidin-3-bioside)	<i>Papaver rhoeas</i>	Scarlet	Pink
Oxidation of anthocyanin aglycone	<i>Cheiranthus cheiri</i>	Purple	Pink
Oxidation and methylation of anthocyanin* (3-monoside)	<i>Primula sinensis</i>	Red	Coral
Local pH changes	<i>Primula sinensis</i>	Magenta	Blue

In addition glycosidal and methylation (without oxidation) changes are probably controlled by simple gene changes, as are acylation changes. The above list is somewhat misleading in that it does not give an idea of the interaction effects which may be either additive or more complex. For instance, the gene-controlled difference between magenta and red in *Primula sinensis* may, on another genetic background, be expressed as a difference between red and slaty. A point of particular interest is that there appears to be competition for a common precursor in the case of certain pigments (see also Lawrence & Scott-Moncrieff). Thus, different anthoxanthins, different anthocyanins, or anthoxanthins and anthocyanins, may show such competition. On the basis of genetic results having to do with such interaction effects, it is suggested that one-half of each competing pigment molecule is derived from a common precursor. This theory finds some support in the chemical work of the Robinsons, according to Scott-Moncrieff (2). Certain qualified generalizations regarding dominance are suggested by Scott-Moncrieff (1) as follows:



<i>Dominant</i>	<i>Recessive</i>
Plastid pigmentation	Absence of
Copigmentation	Absence of
Anthoxanthin pigmentation	Absence of
General and specific anthocyanin pigmentation	Absence of
More oxidized anthocyanins	Less oxidized
More methylated anthocyanins	Less methylated
3-5-diglycosidic anthocyanins	3-monoglycosidic
Acylated (complex) anthocyanins	Simple
More acid petal sap	Less acid

Störmer & Witsch have studied the development of flower-pigment patterns in the *Petunia*. Here no anthocyanin precursor is detected in the white areas of blue and white flowers, but it is detectable in blue areas in 4 to 5 mm. flower buds. A gene pair is known in this plant which controls the transformation from precursor to anthocyanin.

In the case of flower pigments more is known concerning the biochemical and physiological bases of genetic differences than in any other group of hereditary characters.

#### EYE-PIGMENT DEVELOPMENT IN INSECTS

Hormones are known to act as intermediaries between gene and character in a number of instances. In the meal moth, *Ephestia kühniella*, the gene *a* is concerned with a number of differences in pigmentation [see Kühn; and Becker (2)]:

	<i>Dominant (a<sup>+</sup>)</i>	<i>Recessive (a)</i>
Larval eyes	Strongly pigmented	Weakly pigmented
Larval skin	Reddish	Colorless
Testis sheath	Reddish brown	Colorless
Eyes of adult	Black	Red

That these differences are associated with a hormone difference was first shown by Caspari. An *a<sup>+</sup>* larval testis, transplanted to an *a* host, leads to a modification of the pigmentation of all parts listed above, e.g., the eye of the host imago becomes black. In the same way Plagge (3) showed that the hormone concerned in this difference is produced by *a<sup>+</sup>* eye tissue. It can likewise be produced by brain tissue of *a<sup>+</sup>* animals. The time relations for production and utilization of the hormone, known as *a<sup>+</sup>* hormone, have been studied by Plagge (2). Becker (1) has shown that *a<sup>+</sup>* hormone is

soluble in water, alcohol, and 70 per cent acetone. The formation of  $a^+$  hormone is in some manner blocked in genetically  $a/a$  animals.

Following an indication in experiments on mosaics made by Sturtevant that a diffusible intermediary is concerned in the production of eye pigmentation in *Drosophila melanogaster*, Ephrussi & Beadle (1) [see also Beadle & Ephrussi (1)] were able to demonstrate the hormonal nature of this diffusible principle. The results are similar to those outlined above for *Ephestia* except that in *Drosophila* neither brain tissue nor gonads appears to produce the hormone, called  $v^+$  substance, or  $v^+$  hormone. A second eye-color hormone is known, the presence of which is controlled by the cinnabar gene [Ephrussi & Beadle (1)]. This hormone, called  $cn^+$  hormone, is closely related to the  $v^+$  hormone developmentally, and it has been postulated by Beadle & Ephrussi (1) that the two are related sequentially in a chain reaction. Both  $v^+$  and  $cn^+$  hormones are produced by developing eyes [Ephrussi & Beadle (2)]. Both can be obtained from Malpighian tubes, but  $v^+$  hormone alone is produced by transplanted fat bodies of wild type larvae [Beadle (2)]. Eye implants of certain genetic types produce limited amounts of  $v^+$  hormone, e.g., bar (Steinberg & Abramowitz; Chevais, Ephrussi & Steinberg). Ephrussi & Chevais (1, 2) have shown that there is an inverse relation between the amount of pigment formed by an eye implant and the amount of eye-color hormone released. This relation is interpreted by assuming that the hormone produced by the developing eye is used in its own pigmentation, and only the excess is released. If pigment production is limited genetically by making the proper gene substitutions, the amount of hormone released by an eye implant can be correspondingly increased. Time relations in the production of the two eye-color hormones have been studied by Beadle, Clancy, & Ephrussi and by Harnly & Ephrussi. Work by Khouvine & Ephrussi, and by Thimann & Beadle has shown that both *Drosophila* hormones are water- and alcohol-soluble, ether- and chloroform insoluble, and heat-stable. Beadle & Law have shown that both hormones can be effectively administered with food. Chevais found that the addition of  $v^+$  hormone results in an earlier appearance of pigment in genetically vermilion eyes than in controls.

Ephrussi & Harnly have shown that hormones capable of modifying the eye color of vermilion and cinnabar mutants in *Droso-*

*phila melanogaster* can be found in *Calliphora* (Diptera) and in *Galleria* (Lepidoptera). Howland, Glancy & Sonnenblick showed that different species of *Drosophila* contain similar hormone systems. It has been demonstrated by Plagge (1) that the hormone effective in *Ephestia* can be found in moths of other genera. Finally, as the result of the work of Becker & Plagge, of Plagge & Becker, and of Beadle, Anderson & Maxwell, it is known that the eye-color hormones of *Ephestia* and *Drosophila* are physiologically interchangeable, as are those of *Habrobracon* (Hymenoptera) and *Drosophila* (Beadle, Anderson & Maxwell). These physiological similarities among various insects lead to interesting inferences concerning gene homologies. Kikkawa has demonstrated that pigment reactions in the silkworm are also under gene control, and it seems probable that a similar hormone-pigment system is involved here. Unfortunately, only limited information is available as to the chemical nature of the pigments involved in these various insects. Mainx has found that there are at least two pigment components in the eye of *Drosophila*, one red and water-soluble, the other brown, insoluble in all ordinary solvents, and probably bound with a protein. A histological study of eye-pigment development in *Drosophila pseudoobscura* has been made by Cochrane (1) and her results can be correlated with those of Mainx. Cochrane (2) has likewise studied the developmental and genetic relationships known to exist between eye color and testis-sheath color in *D. pseudoobscura*.

From results of interspecific eye transplants, Howland, Glancy & Sonnenblick, Tan & Poulson and Gottschewski & Tan (1, 2) have drawn conclusions concerning eye-color gene homologies between *Drosophila melanogaster* and *D. pseudoobscura*. For example, vermilion and orange of *pseudoobscura* are shown to be homologous with vermilion and cinnabar respectively of *melanogaster*.

Reviews of the developmental and genetical aspects of eye pigmentation in the various insects mentioned above have been written by Kühn, by Becker (2), and by Ephrussi.

#### GENETIC CONTROL OF MELANIN PIGMENTATION

Coat colors in mammals are attributed to melanins or melanin-like pigments. In many species, for example, in mice, rats, guinea pigs, rabbits, cattle, etc., genes are known which control melanin production and distribution. Danneel has pointed out that the

evidence indicates at least three reactions involved in pigment formation in the rabbit: (a) a reaction sensitive to x-rays, (b) a reaction strongly influenced by small fluctuations in temperature, and (c) final pigment formation, which can be inhibited with hydrogen cyanide. Studies on the *a* series of alleles (*A*, colored; *a*<sup>n</sup>, Himalayan-colored extremities; and *a*, white) have indicated an interpretation of dominance in terms of relative rates of enzyme formation and destruction. Danneel & Schaumann have made a number of experiments on the physiology of pigment formation in rabbits differing in the *a* gene. A substance inhibiting the enzymatic formation of pigment from tyrosine or dihydroxyphenylalanine is found in the skin of wild rabbits. The enzyme concerned in this process of pigment formation is not found in the skin of albino (*a*) rabbits or in the belly skin of the wild rabbit (white fur-bearing). The enzyme and the inhibiting substance can be separated. In the Himalayan rabbit, enzyme formation and subsequent pigment formation take place following local cooling of the skin, but even after prolonged "undercooling" not as much enzyme is formed as in *A* individuals. Neumann has also studied the hair pigments of different rabbit races and concludes that black, brown, and yellow melanins are so much alike chemically that they must have a common source.

Following methods developed by Einsele, Daniel has made a spectrophotometric study of hair melanins in the mouse, and finds that those from different genetic types are essentially the same and that differences in coat colors are probably referable to quantitative differences in melanin. Dunn & Einsele, in a study of the hair melanin in mice differing in members of the albino series of multiple alleles, conclude that reduction in quantity of pigment is probably associated with reduction in size of pigment granules. A study by Russell of the albino series in the guinea pig indicates that, in the lower members of the series, there is about twice as much pigment formed in animals homozygous for active alleles as in animals which are heterozygous. On the other hand, the highest allele appears to show complete dominance. This suggests that, with the lower alleles, some reaction in pigment formation is limited by a gene product, but with the highest member of the series, this product is not the limiting factor. Bogart & Ibsen report studies of hair and skin pigmentation in cattle, where several genetic factors are known.

Twitty has shown that pigment amounts and patterns in developing salamanders are influenced by genetic factors and that the constitutions of melanophores themselves are important in determining their characteristic migrations. A genetic factor controlling melanophore formation is reported in the paradise fish by Goodrich & Smith, and the chromatophore reactions in this mutant type were compared with those in the normal type by Dalton & Goodrich.

Gordon has extended studies earlier made by himself and by Kosswig on the genetic control of melanotic tumor formation in top-minnow hybrids involving the platyfish and the Mexican swordtail. It is shown that macromelanophores are necessary for tumor formation in the hybrids; micromelanophores are indifferent in this respect. Additions to his earlier studies of the behavior of color genes in interspecific hybrids among the top-minnows have been made by Kosswig.

Schlottke has published studies of the formation of body pigment in the wasp *Habrobracon*, and Schuurman has reported studies on melanin formation in a beetle of the genus *Tenebrio*. Genetic types in *Tenebrio* are known that show no differences in chromogen, but that do show differences in the enzyme system involved in pigment formation.

#### HYBRID VIGOR

Hybrid vigor or heterosis is of the greatest importance to the plant and animal breeder. Its genetic basis has been the subject of much experimental work and a great deal of speculation [see East (2) for review of recent literature]. Sprague has reported the results of experiments designed to test the suggestion of Ashby that in certain crosses of inbred lines of maize, hybrid vigor is nothing more than the maintenance of an initial advantage in embryo size. Sprague concludes that Ashby's interpretation is untenable even for the same strains of maize originally used by him. This conclusion is in agreement with that of East, who presents numerous evidences that Ashby's interpretation, even if correct, cannot be generalized. As a result of a recent study of hybrid vigor in tomatoes, Ashby maintains that his original interpretation applies in this plant.

Following the recent development of methods of controlling sexual reproduction in various yeasts, Winge & Laustsen have

made hybrids between the two species *Saccharomyces ellipsoides* and *S. validus*. The  $F_1$  generation hybrid was more vigorous than either parent. There was a decline in vigour in the  $F_2$  generation. This work is of significance in that it lays the basis for precise genetic control of an organism that has been of especial interest to physiologists.

Patrushev has made biochemical studies of the blood of the Bactrian camel, of the dromedary, and of the  $F_1$  hybrid between them. He finds a higher glutathione content, a greater catalase activity, and other differences in the hybrid which he attempts to correlate with heterosis. A somewhat similar study is reported by Kushner for cattle, yaks, and their hybrids.

#### SIZE AND FORM

Sinnott (2) points out that the size distribution in the  $F_2$  generation of certain crosses in *Cucurbito pepo*, when plotted on an arithmetic scale, is positively skewed but that these distributions are much more nearly symmetrical when plotted on a logarithmic scale. Sinnott suggests that this type of distribution results from the fact that processes, independent genetically and developmentally, are concerned and act in a multiplicative fashion. As a result of their studies of size inheritance in the tomato, MacArthur & Butler have concluded that fruit size fundamentally involves geometric processes, a conclusion essentially similar to that of Sinnott (2). Sinnott (1) has summarized earlier work on developmental relationships in this same plant and concludes that genes concerned with growth often control growth relations rather than absolute rates. In many gourds growth is heterogenic, *i.e.*, growth rates in two dimensions maintain the same relation to one another over long periods of development; this is expressed by the equation  $y = b x^k$ , where  $y$  represents one dimension and  $x$  the other.

The two related species of *Drosophila*, *pseudoobscura* and *azteca*, differ in testis shape. In *pseudoobscura* the testis of the imago is ellipsoidal while in *azteca* it is spiral. Stern finds that in interspecific testis transplants, the form of the testis implant, when normally attached to the ducts, is determined by the host, *i.e.*, a *pseudoobscura* testis developing in an *azteca* host is spiral. Testis growth is evidently under the control of an inductor-like influence, and this in turn is presumably under the control of the genotype.

Van Overbeek (1) has presented further studies of the relation of the growth hormone, auxin, to genetic dwarfing in maize. Seedling tissues of the recessive type *nana* inactivate auxin more rapidly than do tissues of normal plants. This appears to be a case of genetic control of an oxidation-reduction system. Van Overbeek (1) found that a number of other genetic dwarf types in maize produce auxin at a lower rate than do their normal sibs.

Geotropic responses in plants are known to be related to auxin distribution. Van Overbeek (2) has made a physiological study of the genetically recessive type in maize known as "lazy." Lazy plants assume, when partly grown, a prostrate growth habit. Shafer (unpublished work cited by Van Overbeek) and Van Overbeek have related this lack of geotropism (in some cases apparently a slight positive geotropic response) to auxin distribution. In horizontal normal maize plants auxin becomes more concentrated in the lower half of the stem and results in a growth-produced upward bending. This unequal distribution does not occur in horizontal stems of "lazy" plants. A similar "lazy" character has been reported in rice by Jones & Adair. "Lazy" rice plants, differentiated from normal by a single gene, are slightly or not at all geotropic.

Genetical and histological studies of self-sterility in various plants indicate the existence of many highly specific physiological factors concerned with pollen-tube growth. Recent genetic studies of this phenomenon, first genetically analyzed in *Nicotiana* by East and coworkers, include those of Gruber & Waldenburg; Tseng; and Emerson. Genetic interpretation in each of these cases assumes a series of multiple alleles,  $S^1$ ,  $S^2$ ,  $S^3$ , etc. The genetic rule is that pollen-tube growth is poor where an allele is common to the haploid pollen grain and the diploid style in which it grows; thus, neither  $S^1$  nor  $S^2$  pollen tubes grow normally in  $S^1/S^2$  styles. On the other hand,  $S^3$ ,  $S^4$ ,  $S^5$ , etc., pollen grains grow perfectly in  $S^1/S^2$  styles and  $S^1$  and  $S^2$  grains grow in  $S^3/S^4$  styles. An analysis of the physiological basis of these relations is much in need, for there appears to be a good chance that here the relation between gene and character may be rather direct. In addition to the  $S$  series of alleles known in a number of plants, there are other genetic factors known to influence pollen-tube growth. Burnham and Brieger have each recently reported such genetic factors in maize.

A single gene substitution in *Hyocymus niger* (Solanaceae) is



capable of making the difference between an annual and a biennial growth habit. Genetically biennial plants can be induced to flower in one year by appropriate low temperature treatments. Melchers has shown that the flowering response can be transmitted across a graft union from tissue of a genetically annual to a genetically biennial plant. Melchers interprets this on the basis of a relation, probably not direct, between genetic constitution and the action of a non-species-specific hormone for the stimulation of flower production. Other investigators, cited by Melchers, have presented evidence that such a hormone exists. Attempts of Melchers to apply the hormone in the form of extracts were unsuccessful.

It has been reported by Wettstein & Pirschle that a recessive character, *defecta* (*d*), in *Petunia* differs from normal by a hormone-like substance that can move across a graft union. *Defecta* plants have small flowers, small leaves differing from normal in shape, and are deficient in chlorophyll. A *defecta* scion is strongly modified in some respects when grown on a genetically normal stock. Further work should indicate more precisely the nature of the diffusible principle here concerned.

Wings of insects have long been an object of study in problems concerning the physiological basis of form and pattern. Citations to earlier work in this field can be found in Goldschmidt's recent book. Goldschmidt (1) has continued his studies of wing shape in *Drosophila*. He has shown that among different vestigial alleles, the wings are normal up to a certain time of development and that thereafter local degeneration and resorption sets in. The final size and shape of the wing, according to Goldschmidt, is dependent on the time at which retrogression sets in, which in turn, can be ascribed to gene-controlled differences in rate of production of a hypothetical differentiating or, alternatively, wing-destroying substance. Blanc & Child have reported studies on the relation of temperature shock to the so-called truncate reaction in *Drosophila*, a reaction concerned in the production of a definite wing type genetically differentiated from normal. Henke (3) has reported detailed studies of external and genetic factors influencing wing size and shape in *Ephesia kühniella*.

Ephrussi, Khouvine & Chevais and Chevais & Steinberg have reported that by feeding nitrogenous extracts of *Calliphora* pupae to larvae of bar-eyed *Drosophila melanogaster*, the number of facets can be greatly modified, increasing with the concentration of the



extract fed. Presumably such extracts contain a specific substance concerned with facet formation. This substance is distinct from  $v^+$  hormone, also contained in the *Calliphora* extracts used, according to the results of Chevais, Ephrussi & Steinberg.

Many genetically non-allelic "minutes" (bristle characters) are known in *Drosophila*. The genes (deficiencies in a number of cases) that differentiate them from normal are dominant and lethal when homozygous. Dunn & Mossige and Brehme (1) have recently investigated developmental rates in "minutes" as compared with normal flies and have found that development is prolonged in the larval stage. Brehme (2) has studied the time of death in three "minute" homozygotes and finds it to occur mainly in the first instar.

#### LETHALS

Many so-called lethal genes are known in the various organisms that have been extensively used for genetic studies. In a number of instances, attempts have been made to determine the physiological and developmental bases of lethality. Among the more recent work is found that of Hadorn (1, 2, 3) on the genetic type "lethal-giant-larvae" (*lgl*) in *Drosophila melanogaster*. Larval organs are more or less normal, but imaginal ones are abnormal or do not develop at all. Hadorn showed that ovaries from *lgl* larvae transplanted to normal larvae will develop considerably beyond the stage they would have reached had they remained in the original environment. Another characteristic of *lgl* larvae is that they either do not form puparia or form them much later than do normal sibs. Hadorn has shown that puparium formation may be induced in *lgl* larvae by transplanting to them a larval "ring-gland" from a normal larva. They do not, however, undergo further development. Poulson has reported studies on the embryological irregularities in *Drosophila* embryos deficient in sections of various lengths of the X chromosome. It is found that the larger the chromosome deficiency, the earlier and more general are the causes of abnormal development. McClintock has correlated specific developmental abnormalities in maize with specific chromosomal deficiencies in studies of mosaic plants. The above and related studies in *Drosophila* and other organisms (e.g., those of Demerec and coworkers) agree in indicating the fundamental importance of most genes in developmental processes.

Grüneberg (1) has continued his studies of the "grey lethal"

in the mouse. The many apparently diverse effects of this recessive mutant, with the possible exception of a pigment anomaly, appear to be consequences of a lack of secondary bone absorption. Grüneberg (2) has likewise studied a "pleiotropic" lethal mutation in the rat and finds that it is possible to ascribe the several characteristic developmental aberrations to a cartilage anomaly.

#### SEX-DETERMINATION

Among the unicellular ciliates such as species of the genus *Paramecium*, conjugation involving a kind of reciprocal fertilization is a common phenomenon. The conjugants of *Paramecium* are not morphologically different. Each contributes a stationary pronucleus, comparable to the female contribution in higher animals, and a migratory pronucleus, comparable to the male contribution in higher animals. In one sense these organisms are hermaphroditic. Sonneborn (1, 2) [see also Sonneborn & Lynch, and Kimball] has shown that individuals of *Paramecium aurelia* may be differentiated into two types with respect to sex reactions. Two individuals of one type will not conjugate, but under appropriate conditions two individuals of different types regularly undergo conjugation. The genetic basis of this differentiation has been shown to be simple, and in many respects similar to that in higher animals. While there appear to be only two reaction types with respect to conjugation within a given race of *Paramecium aurelia*, Jennings (2, 3) has shown that in *Paramecium bursaria* there are many reaction types, the members of any one of which do not conjugate with one another, but which do conjugate with members of other types. Jennings (3) suggests that two definite sexes may have evolved by gradual specialization among many reaction types.

#### EXTRANUCLEAR AND MATERNAL FACTORS IN INHERITANCE

In a study of the inheritance of the characteristics of the teeth of the vegetatively reproducing rhizopod, *Diffugia corona*, Jennings (1) found that by making the mouth of a parent irregular by means of operations, the mouth and surrounding teeth of the progeny were irregular, but less markedly so than those of the parent. It is as though the characteristics of the mouth and teeth of an individual were determined by some process of induction by the parent. Jennings points out the possibility that the phenom-

enon here involved may be analogous to the phenomenon of persistent modifications (*Dauermodifikationen*) which result from subjecting protozoans to heat, various chemicals, etc. (Jollos). *Dauermodifikationen* have never been satisfactorily interpreted on a genetic basis.

Murray & Little have demonstrated that incidence of breast tumors in mice differed in reciprocal hybrids between mice from "high" and from "low" breast-tumor strains. The extrachromosomal influence postulated to account for these observations appears from the work of Bittner & Little and of Bittner to be due to a "breast-cancer-producing influence" carried in the milk of "high" breast-tumor mothers.

Kühn & Plagge have shown that in *Ephestia* there is an influence of  $a^+/a$  mothers on their immediate  $a/a$  offspring, which can be interpreted as a transfer of  $a^+$  hormone in the egg from one generation to the next. The same end result can be brought about by artificially supplying an  $a/a$  female with  $a^+$  hormone; her immediate offspring then show the effects of  $a^+$  hormone. This is the only case known to the reviewer of a genetic "maternal influence" or "predetermination" being ascribable to a specific known substance transmitted through the egg.

Several recent reviews of the subject of cytoplasmic inheritance and its relation to the nuclear system are available [East (1); Goldschmidt (5); Wettstein; Plagge (4); Sirks; and Michaelis].

#### ARTIFICIAL INDUCTION OF POLYPLOIDY

The alkaloid colchicine has the curious property of inhibiting spindle formation during cell division while permitting chromosome multiplication to proceed more or less normally. Since the inhibition of spindle formation is reversible, colchicine treatments may result in doubling the chromosome number of a cell. Blakeslee demonstrated that this drug could be effectively used in producing entire tetraploid plants. At about the same time, Greenleaf developed a technique for inducing polyploidy in tobacco through treatment with indole-3-acetic acid (heteroauxin). Although methods of artificial production of polyploids had previously been developed, e.g., Randolph's heat-treatment method, the colchicine technique, because of its convenience and certainty, is potentially of great significance in plant breeding. Reports on the action and use of colchicine in this connection have been given by Blakeslee

& Avery; Blakeslee, Avery, & Cartledge; Nebel (1, 2, 3); Nebel & Ruttle; Eigsti; Levan; Dermen; and by Walker. Following Shmuck's prediction, made on chemical grounds, that it should be similar to colchicine, Kostoff (1, 2) and Navashin have found that acenaphthene is effective in inducing polyploidy and other changes in chromosome number in plants. Its action appears to differ in detail from that of colchicine, in fact, Nebel (3) failed to obtain any polyploids in a preliminary test of acenaphthene.

It has several times been suggested that the atypical growth associated with tumors of various kinds, both in plants and in animals, is related to irregularities in chromosome numbers and behavior. Wipf & Cooper have made the significant observation that bacterially infected nodule cells in red clover, common vetch, and garden peas are regularly tetraploid whereas normally these plants are diploid. No interpretation has yet been offered of this rather striking observation. In this connection, Jones has considered the general question of irregularities in chromosome behavior and atypical growth.

#### GENETIC CONTROL OF AGGLUTINOGENS

The genetic basis of blood-group differences in man and other animals has been understood for a number of years. Making use of standard immunological techniques for working with blood-cell agglutination reactions, Irwin & Cole, and Irwin (1, 2) have continued earlier studies of various species of doves and pigeons and their hybrids. The four species, Ring Dove, Pearlneck Dove, common pigeon, and triangular spotted pigeon, were studied. It has been found that each species has agglutinogens not present in any one of the others. Comparing any two species, it is found that each has its specific agglutinogens as well as a group common to the two species. In certain hybrid combinations there appear "hybrid" agglutinogens, *i.e.*, those not found in either parent, but found in the  $F_1$  birds. These presumably arise through gene interaction. In addition to these hybrid substances, there are, in the hybrids, the components shared by the two parents and most of the components not shared. Both species-specific and common antigens have been shown to be under gene control. In backcrosses of the  $F_1$  hybrid between Ring Dove and Pearlneck to the Ring dove parent, ten different antigens peculiar to Pearlneck

have been identified. In such backcrosses each shows a simple 1:1 segregation. The hybrid substances can be "fractionated" by such backcrossing.

#### MUTATION

With the recent advances in our knowledge of viruses, there has been a renewed interest in comparisons between them and genes. Gowen & Price and Gowen have compared the x-ray dosage inactivation curves obtained with tobacco virus and those obtained for the effects of x-rays on genes and found them to be essentially similar. Other striking similarities between viruses and genes have been pointed out by these and other authors, for example, the property of mutation (McKinney; Salaman; Findlay).

Within the past few years there have been a number of reports on the production of mutations in *Drosophila* and other organisms by means of various chemical treatments. Many of these studies have been unsatisfactory in one respect or another; e.g., in many cases the effects are little more than barely significant statistically. Stubbe and Lobashov have each published summaries of work on the chemical induction of mutations. Sacharov & Naumenko find that chemically induced lethal gene mutations in the X chromosome of *Drosophila* are not cytologically distinguishable from spontaneous ones.

Two interesting reports have appeared on the effects of nutritive conditions in plants on gene mutation. Döring found that *Antirrhinum majus* plants grown in water cultures and deprived of necessary elements, or grown at an unfavorable pH, showed a mutation rate of the order of three times that of normally cultured controls. Schlösser grew *Lycopersicum cerasiforme* plants under crowded conditions and with a deficiency of nitrogen, and found a greatly increased mutation rate over that of the controls.

Plough & Child report increases of three to six times in the rate of spontaneous lethal mutations in the second and third chromosomes of *Drosophila* following twenty-four hour high temperature treatments (36.5°C.). Medvedev has made the observation that, with a 4000 r unit x-ray treatment, the lethal mutation rate for the X chromosome of *Drosophila* is higher at 19 to 20°C. than at 36 to 39°C. This observation is at variance with others indicating that there is no temperature coefficient for the x-ray effect on mutation rate. In this connection, Mickey reports about twice as many chromosome translocations following x-ray treat-

ment of *Drosophila* males at 4°C. as at 28 to 30°C. The significance of these unexpected results is not known.

Catcheside (1, 2) has attempted, through cytological studies of x-ray induced chromosome aberrations in *Drosophila* and in maize, to distinguish between the "breakage" hypothesis (independently induced breaks followed by random reattachment of the resultant fragments) and the "contact" hypothesis (chromosome or chromatid fusion followed by breakage along a new plane, i.e., "illegitimate" crossing over), but was unable to do so from the data obtained. Sax finds that x-ray induced chromosome aberrations in *Tradescantia* (treatment near or at meiosis and examination of subsequent microspore divisions) increase in frequency geometrically with dosage. This appears not to be in agreement with the observations of Catcheside (1) and others who find a linear relation between chromosome aberrations and dosage. As indicated by Sax, however, most of the aberrations observed by him are eliminated within a few cell generations, while those on which Catcheside bases his conclusions correspond to the survivors. Furthermore, as Catcheside (1) points out, the type of dosage curve obtained will depend on the dosage range covered; Sax worked at lower dosage levels than did Catcheside. Kaufmann & Demerec find from cytological analyses of salivary gland chromosomes of *Drosophila* that x-ray induced chromosome breaks occur equally often per unit length in heterochromatic Y and in euchromatic autosomes. This suggests that the basic structure of chromosome threads is similar in both genetically "active" and genetically "inactive" regions.

Stadler & Sprague, Stadler, and Stadler & Uber have reported that through comparisons of relative frequencies of various x-ray and ultraviolet effects in maize, alterations resulting in translocation are separable from those resulting in point mutations, deficiencies and germless seeds ("dominant lethals"). Those producing germless seeds are separable from those producing deficiencies.

Slizynski has made a cytological comparison of x-ray induced and spontaneous X-chromosome lethals in *Drosophila* and finds no evidence of a difference in the relative frequencies of detectable deficiencies in the two classes. This is in disagreement with earlier work cited by Slizynski.

Timoféeff-Ressovsky has reviewed the general question of the

experimental study of gene mutations and chromosome aberrations.

With the appreciation of the significance of the giant salivary chromosomes of *Drosophila*, it has become increasingly difficult to draw a distinction between so-called point mutations and chromosome aberrations (Sokolow; Sax; and others). Many very small chromosome aberrations have been reported. At the same time more and more attention has been given to the phenomenon known as "position effect," the concept that properties of a genetic system are determined not alone by the characteristics of the individual genes that make it up, but as well by their spacial relations with respect to one another (see Dobzhansky for reviews of literature). In addition to the several completely established cases of position effect known in *Drosophila*, many probable ones have been reported, e.g., Muller & Raffel and others. Grüneberg (2) has reported a reinversion of the roughest inversions in *Drosophila* accompanied by a reverse "mutation" of roughest. Since, according to Grüneberg (2), this reversion of roughest was not detected until about 85 per cent of the stock-culture flies showed it, there is room for reasonable doubt concerning this particular alleged proof of position effect. Caspersson & Schultz have made the interesting observation that the nucleic acid content of individual bands of the salivary gland chromosomes of *Drosophila* appears to be related to their positions with respect to the heterochromatic regions, i.e., if bands are placed close to such regions, their nucleic acid content is increased. A relation of this to genetic position effect is suggested. It is also suggested that there is a relation between nucleic acid and gene reproduction.

With the discovery of a number of instances of position effect, it has been suggested by Goldschmidt (2, 3, 4, 5) that all mutations may be merely position effects. On the basis of spontaneous "mass" mutations in *Drosophila* associated with chromosome aberrations, Goldschmidt proposes the complete abandonment of the gene concept—"In other words, there are no genes, no gene mutations and no wild type allelomorphs." He would substitute position effect for all heritable changes in the chromosomes. With regard to the so-called mass mutations, it should be pointed out that "genes" are known which increase the general rate of spontaneous mutations of other "genes" [Beadle (1); Demerec]. Plough & Holthausen may also have been dealing with such a



mutation-stimulating mutant in their reported case of abnormally high spontaneous mutation rate in *Drosophila*. Recently, Rhoades has reported studies of a gene in maize which greatly increases the mutability of the particular color gene  $a_1$ . It is possible that Goldschmidt's mass mutations may have resulted from some such genetic condition particularly favorable to the production of mutations. However this may be, the reviewer finds it extremely difficult to reconcile the views of Goldschmidt with many of the well-established facts of modern genetics. A final decision as to the tenability of these views, however, must await a more detailed elaboration of them in terms of the basic data of genetics, and the presentation of complete data on the nature of the chromosome aberrations associated with the reported mass mutations.

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SCHOOL OF BIOLOGICAL SCIENCES  
STANFORD UNIVERSITY,  
CALIFORNIA

## DEVELOPMENTAL PHYSIOLOGY\*

BY JOSEPH NEEDHAM

*Gonville and Caius College, Cambridge, England*

Embryology was for too long a time the perquisite of morphology. Yet the development of function is inseparable from the development of form. The development of form, indeed, depends on the normal progress of the metabolic changes in the embryo, for as we now know, much of differentiation is dependent differentiation. Morphogenetic hormones or stimulating substances do not arise from nowhere, but are themselves chemical entities, with a metabolic origin and a metabolic fate. So also are the protein molecules which form the architectural basis of morphology. Developmental physiology includes experimental embryology (*Entwicklungsmechanik*) and chemical embryology. Genetical studies, too, have recently become important in this connection, since the many inheritable aberrations or modifications of development throw light on its machinery.

### DETERMINATION PROBLEMS: THE MORPHOGENETIC STIMULI

The greatest progress in recent times has been made in the cases of the amphibia and the echinoderms.

We know that the primary organiser of the amphibian embryo, which at gastrulation stimulates the dorsal ectoderm to form the neural tube (the main vertebrate axis), involves the action of a chemical stimulus exerted by the archenteron roof. [Recent reviews by Spemann (2); Brachet (2, 4); Needham (3, 4); Lehmann (1, 3); Holtfreter (3); Bytinski-Salz (1); Brøndsted; Seidel (1, 2); Woerdemann (1); Daniel]. We know that the organiser-hormone (evocator) is present throughout the egg, but liberated at the organiser centre (dorsal lip of the blastopore). Ventral ectoderm, if killed, as Holtfreter (1) showed, becomes inducing, *i.e.*, can act like the dorsal lip. Yet ventral ectoderm is the only available tissue on which to test inducing power. Inductors may therefore sometimes act indirectly, by liberating the tissue's own evocator, as well as directly, by acting themselves similarly to the natural evocator. Dosage studies may distinguish between these two possibilities: the smaller the amount of substance required, the more

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likely it is to be acting directly. The neural inductions of Fischer *et al.* with nucleoprotein and acid preparations are therefore probably indirect effects, as also must be those of Barth with very acid or alkaline jellies. Some very weak effects were found by Okada on implantation of kaolin and other inorganic substances; they were accompanied by cell-degeneration, so that the same explanation probably holds. In the production of double monsters by centrifuging (as by Schechtman), competent ventral ectoderm is probably brought into contact with inductors which it never normally meets. Plant substances of all kinds continue to give absolutely negative results, as the photographs of Ragozina and of Toivonen show.

Preliminary evidence that the primary organiser is steroid in nature was supported by powerful effects produced by many new polycyclic carcinogenic and oestrogenic hydrocarbons, such as styryl blue, methylcholanthrene, dihydroydiphenyl, and dinaphthylacenaphthene in Waddington's implantations. Shen, using the water-soluble carcinogenic hydrocarbon, 1, 2, 5, 6-dibenzanthracene- $\alpha$ ,  $\beta$ -endosuccinate, implanted in carefully graded doses, obtained a maximal activity in neural induction at 0.007  $\mu$ g. per embryo. Massive neural palisades were still found at a dose of 0.0001  $\mu$ g. per embryo. This range of doses is comparable with that of many hormones and vitamins; it strengthens the evidence that hydrocarbons act directly, not indirectly, on the ventral ectoderm.

The mechanism of the liberation of the evocator from its inactive precursor or complex in the dorsal lip will only be understood in the light of a knowledge of the metabolic differences between this active centre and the rest of the gastrula. By use of the Cartesian Diver ultramicromanometer of Linderstrøm-Lang, it has been found that the anaërobic glycolysis and ammonia production of the dorsal lip region is three times as high as that of the ventral ectoderm (Needham, Boell & Rogers), but that the oxygen consumption is the same in the two regions (Boell & Needham, in agreement with Waddington, Needham & Brachet, but contrary to Brachet & Shapiro and to Fischer & Hartwig (2) who find a slight difference in  $Q_{O_2}$  in favour of the dorsal lip). The respiratory quotient, studied by Boell, Needham & Koch, in the neighbourhood of 0.7 in the blastula roof and 1.0 in the closing neural folds, showed a tendency to rise quicker and further during gastrulation in the dorsal lip region than in the ventral ectoderm. These results,

which in general agree with the well-known loss of glycogen from the invaginating material, suggest that there are profound metabolic changes in the dorsal blastopore lip but rather of quality than of quantity. Following earlier work by Fischer & Hartwig (1), Piepho reports that if gastrulae are stained vitally with reducible dyes, anaërobic decolorisation proceeds fastest in the dorsal lip region. Brachet (5) amplifies this by finding the highest fixed -SH concentration (nitroprusside test on unpigmented embryos after trichloroacetic acid fixation) in the dorsal lip region.

The action of dyes in liberating the evocator from isolated pieces of ventral ectoderm is not confined, according to Beatty, de Jong & Zieliński, to redox indicators known to increase respiratory rate, although methylene blue does to a small extent accelerate respiratory rate of ventral ectoderm. Needham & Boell found indications that dinitro-*o*-cresol had a greater effect on  $Q_L^{N_2}$  of ventral ectoderm than of dorsal lip, but Finkelstein & Schapiro obtained no neural inductions by implantation of dinitrophenols. Dinitrophenol, applied to intact developing embryos by Dawson and Buchanan, showed only a moderate effect inhibiting developmental rate.

Destruction of the inducing power of dorsal lip pieces with ultraviolet irradiation was not obtained by Reith till the death of the tissue, suggesting that the cases of invagination after irradiation followed by induction failure observed by Dürken were not due to destruction of the evocator itself. Lehmann (2) has further studied the inhibition of notochord formation caused by lithium application; he terms it a "mesodermisation" (see below). Though the embryo is in every other way practically normal, the notochord is wholly absent, its material having formed somite tissue.

That the evocator is present in both nucleus and cytoplasm of the unfertilised egg has been shown by Waddington (2).

So far the chemical component in neural induction has alone been spoken of. But we know that the organiser region possesses regionality, that is, living head organiser will not perform quite the same inductions as tail organiser. It was for this reason that the terms "evocation" (the chemical stimulus for neuralisation) and "individuation" (the field system of headness and tailness), were invented. Spemann's original experiments (1) showed that head organiser would produce head inductions whether implanted in head region or in tail region, but that tail organiser would produce

tail inductions in tail region and head ones in head region. There was thus a head "dominance." But Holtfreter (2) has now shown in beautiful experiments that if head and tail organisers are implanted into isolated ectodermal balls (*i.e.*, free from the individuation field of an embryo) they always work specifically, *i.e.*, the reacting tissue has no influence on what happens. Isolated ectodermal balls were also used by Heatley, Waddington & Needham for studying pure evocation. Glycogen preparations with evocator adsorbed on them were implanted into such balls, with the formation of chaotic neural masses containing many hollow spaces, not unlike the structures obtained in explanted determined neural plates, *i.e.*, neural tissue removed from influences of neighbouring organs in the field. Holtfreter's work was complemented by that of Hall (1), who by interchanging head and tail organisers in an embryo, inverted the primary embryonic axis (Spemann's original experiments had been made by implantations into the blastocoele cavity, and hence the production of a secondary embryonic axis). In these circumstances, no brain was produced at all, and the anterior end of the embryo resembled an "elephant trunk" owing to neural tube elongation. Conversely the head organiser failed to make a head at the posterior part of the embryo. Short reviews of these complicated but extremely interesting questions have been given by Needham (2) and by Waddington (3). The latter considers the possibility that besides the primary evocator, there may be other substances, "modulators," responsible for regional determination.

After the primary induction of the neural axis, the secondary organisers come gradually into play. Of these the most studied is the lens-inducing action of the eye-cup on the ectoderm, of which a fine account will be found in Spemann's book. In some species the action of the eye-cup is absolutely necessary, but in others the ectoderm may make some attempt at lens-formation even in the absence of the eye-cup. It is now certain that ectoderm is competent to react to the secondary organiser of the eye-cup even if it has never passed through the influence of the primary organiser [implantation of eye-cup into isolated ectodermal balls by Waddington (1); implantation of eye-cups into embryos, from which the primary organiser had been totally removed, by Filatow; implantation of eye-cups under transplanted early gastrula ectoderm by Woerdemann (2)]. Physicochemical analysis of the secondary



organiser of the eye began with Lopaschov (1) who got, not lenses alone, but structures like optic cups and lenses produced by them, when he implanted dead eye-cups into the blastocoele cavity. On the other hand, nasal placodes and ear-vesicles did not produce their like from ventral ectoderm [Lopaschov (2)]. The story was continued by van Cleave, who implanted dead eye-cups into isolated ectodermal balls, and obtained eye-cups together with lenses formed by the action of the latter on the ectoderm. Ether extracts of dead eye-cups were inactive. Absence of any species-specificity in a secondary organiser (like the primary one) is proved for ear induction as between anurans and urodeles by Albaum & Nestler.

When we turn to the development of the sea-urchin, we find that the recent great development of our knowledge has led to a form of presentation rather different from that for the amphibian. Instead of primary and secondary organisers, workers on echinoderm development prefer to speak of a double gradient system, one influence radiating from the animal pole and one from the vegetative. It is unfortunate that no comprehensive review of the work of the Swedish school has yet appeared in English, but the Woods Hole paper of Hörstadius (2) and the symposium which followed it are most valuable. A review of the facts, besides much new experimental work, was given in the long paper of Lindahl two years ago. The essential points are the following. Animal pole material produces ectodermal organs, such as the ciliary tuft and the ectoderm. Vegetal pole produces endodermal organs such as the gut and skeleton-building mesenchyme. By treatment in various ways, one or the other of these differentiations can be caused to suppress its fellow, so that the embryo becomes either "animalised" ("ectodermised") or "vegetalised" ("endodermised"). In the former case no gastrulation may occur and coelom, primary and secondary mesenchyme, and endoderm may fail, while the ciliary tuft tissue may invade the whole. In the latter case ciliary tuft tissue will be suppressed, all the endodermal organs will increase, and in extreme cases exogastrulation may occur so that nearly the whole of the material available turns into endoderm. Now it is important that these effects can be obtained either by transplantations of the blastomere layers [Hörstadius (1, 3)], or by chemical treatments (Lindahl), with absolute correspondence. Thus transplanting the micromeres from the vegetal pole leads to vegetalisation, but so does treatment with lithium ions. Similarly "inductions" by an-

imal pole material may be obtained, but at the same time absence of sulphate ions from the water will produce animalisation, or the treatment of the eggs before fertilisation with iodide or thiocyanate.

Much has been done to elucidate the action of these chemical agents. Lindahl has shown that lithium ions have an inhibitory effect on embryonic respiration, additive with that due to lack of sulphate ions. The lithium-sensitive respiration is that part which normally increases three hours after fertilisation. The lithium effect is increased by cyanide and reversed by pyrocyanin [Runnström (1,2)]. It is believed, not perhaps on entirely convincing grounds, that the lithium-sensitive processes connected with, or characteristic of, animal pole material, are those of carbohydrate metabolism. It is also believed that absence of sulphate ions renders impossible the detoxication of phenolic aromatic compounds presumably produced by protein metabolism in the vegetal pole material; Lindahl certainly shows that phenol-sulphatases are present in sea-urchin embryos. According to Lindahl & Stordal, potassium indoxyl sulphate has an animalising action if used in calcium-free sea water before fertilisation, and lack of sulphate ions increases the amount of differentiation-inhibiting substance given off by embryos developing. Isolated animal pole halves were unaffected by lack of sulphate ions, isolated vegetal pole halves were worse affected than usual. Lindahl & Öhman find different temperature coefficients for the lithium-sensitive and -insensitive portions of respiration. At higher temperatures than normal the lithium effect on morphogenesis is intensified. Further experiments with egg-breis are reported.

If we compare the double gradient system of the sea-urchin with the organiser system of the amphibian, the difference is less great than at first might seem, since in the amphibian also there is an endodermal determination, usually overshadowed in our thought by the spectacular "endocrine" effect of archenteron on dorsal ectoderm. In exogastrulation experiments all endodermal organs are perfectly formed, though inside out. One might almost say that the vast yolk accumulation of the amphibian egg, compared with that of the sea-urchin, keeps the two poles much further apart than in the latter, so that their interaction is difficult to see. Such a comparison has been made by Dalcq and Ubisch.

Sea-urchin development is even more difficult to study than

that of amphibia, and as the eggs are so small, it has been inevitable, though unfortunate, that much of the work has concerned the effect of chemical substances on development rather than the metabolic processes involved. Attempts to get over this difficulty are being made by recourse to histochemical techniques [Ranzi & Falkenheim; Ries (1, 2, 3); Ries & Gersch; Gersch & Ries] but relatively little progress is to be expected from them for either vital stains are used, in which case enigmatic polar tint-distributions occur, or else methods of uncertain reliability purporting to show the distribution of glutathione, ascorbic acid, etc., in the cell. In all interpretations of such work caution is necessary. The reduction rate of dyes in different regions of echinoderm embryos, studied by Child, however, merit notice.

Events in the development of fish embryos, at any rate of teleosts, according to Oppenheimer and Luther, follow the organiser type of the amphibian. Lately this has been extended to the lamprey by Bytinski-Salz (2). But in the eggs of insects, as we may learn from the reviews of Seidel (1, 2), an entirely different state of affairs is found. Here we have a very elongated mass of yolk in which at first nothing but repeated divisions of nuclei occur; eventually some of these find their way into one extreme end of the egg, known as the "*Bildungszentrum*" or activation centre. Thence a colloidal change in the yolk spreads over the rest of the egg; in some species it becomes visibly more firm and transparent. On reaching the region of the presumptive prothorax, about half-way along the egg, the "*Differenzierungszentrum*" is brought into action. It starts a contraction of the yolk, leaving a space into which the blastoderm cells migrate. It seems certain, according to the valuable review of Richards & Miller, that the activation centre operates by the diffusion of a chemical substance, while the differentiation centre operates by "dynamic motive processes" which probably depend on molecular contractility of yolk micro-fibrils. Although there is clearly a similarity between activation-centre and amphibian primary organiser, no physicochemical experiments have yet been reported for insects.

#### DETERMINATION PROBLEMS; THE MORPHOGENETIC RESPONSE

When the amphibian dorsal ectoderm moulds itself under stimulus into neural plate and tube, or when the sea-urchin endoderm is forming gut, rearrangement and orientation of protein micelles

must be among the most fundamental processes involved. Much interest attaches therefore to the study of the proteins of embryonic cells. The existence of micro-fibrils oriented in a definite way and reforming after interference might also explain the polarity and symmetry properties so marked in embryonic development [Fauré-Fremiet; Harrison (1, 3); Needham (1)]. It is thus significant that Schmidt and Moore & Miller have observed doubly refracting phases in the protoplasm and nucleus of echinoderm eggs, and that Pfeiffer, deforming molluscan eggs in capillary tubes, describes striated bands indicating cytoplasmic micro-fibrils.

Significant also is Mirsky's work on echinoderm proteins. He disintegrated the eggs by drying at the temperature of solid carbon dioxide, and obtained soluble protein fractions, of which there was more after fertilisation than before, and which showed double refraction of flow. Double refraction of flow was also observed by Böhm & Signer on ovoglobulin from hen's egg white (probably containing much ovomucin), and by Needham & Robinson on livetin of hen's egg yolk.

It has been suggested that the paracrystalline nature of cell constituents may explain the remarkable fact in morphogenesis that the spatial axes of organs, such as limb-buds, are not determined at the same moment of time [Harrison (2)]. New work on the ear in this connection has been done by Hall (2), and Swett found means of hastening or delaying the various axial polarisations of the limb-buds.

#### ENERGETICS, DIFFERENTIATION AND GROWTH

Passing over with but a mention the theoretical paper of Wetzel, which introduces so many new concepts in embryonic growth that it has hardly yet been evaluated, and the interesting criticism directed by Burton against the master-reaction theory, the work of Brody on relativity of physiological time and physiological weight merits notice. Comparison between the growth of widely different organisms is possible if the mid-point between the self-accelerating and the self-inhibiting phases of the cycle is used as central to the frame of reference. As the self-accelerating phases differ among different organisms far more than the self-inhibiting phases, however, this is the less directly important for embryological studies. Glaser proposes a new form of plot for absolute and relative growth during development, the value of which, though

not immediately obvious, should not be ignored. Teissier has further examined the heterogonic or relative growth of chemical constituents in embryonic development and claims the existence of two main points of inflection of such curves (approximately at a wet weight of two and ten grams in the chick embryo). The original suggestion is made by Glaser & Child that the stacking of hexoctahedra takes place according to a law similar to the heterogonic relationship, and they show that such forms exist in cells, but it is a little difficult to see how the regularities of chemical heterogony could be explained on this basis. Thompsonian co-ordinates have at last been applied to embryonic development (by Richards & Riley) but the results so far only cover larval amphibian, not earlier, stages. The developmental pattern is strikingly illustrated.

The series of papers on energetics by Tyler and his colleagues has been amplified by three further works. Sea-urchin eggs developing within tight membranes (the elevation of which has been stopped) have thicker blastula walls than normal and smaller total diameter, but the rate of respiration is the same as that of the normal egg (Tyler). Respiration therefore seems independent of these morphological changes. There is no difference between temperature coefficients of respiration in unfertilised and fertilised eggs (Tyler & Humason). Non-cleaving parthenogenetically fertilised gephyrean eggs show a respiratory rate rising more slowly than those similarly treated which cleave, and these again more slowly than normally fertilised eggs. Normally fertilised eggs which have their cytoplasmic cleavage inhibited and their nuclear division retarded by phenylurethane show less rise in respiratory rate than normal, *i.e.*, they resemble the parthenogenetic ones. In all these cases there is parallelism between cleavage and rise of respiratory rate. In this connection Brachet (3) made interesting observations on *Chaetopterus* eggs undergoing "differentiation without cleavage" after treatment with isotonic potassium chloride. Their respiratory rate rises much less rapidly than that of normally fertilised and properly cleaving eggs, and their thymonucleic acid synthesis is much below normal.

One of the most serious gaps in our knowledge of early development is the exact nature of the cell-streams which bring about the deformations and displacements characteristic of gastrulation and similar processes. Judging from the laborious work of Richards;

Richards & Porter; and Richards & Schumacher on the fish, and Derrick and Teague-Self on the chick, local accelerations of mitotic rate are not responsible. This is also the generally accepted view in the case of the amphibian, but the nature of the process is obscure and too often glossed over by embryological writers. In ascidia the relations between cell-division and histo-differentiation are exceptionally clear, and Berrill has described them in two papers. Genetic differences, too, have been under investigation, and Byerly, Helsel & Quinn have decided that no differences in cell-number or mitotic rate exist between chick embryos differing genetically in size.

#### GENETICS AND DEVELOPMENTAL PHYSIOLOGY

The mechanisms of normal development may often be illuminated by study of cases of abnormal development. Many of these are due to the action of genes. A promising beginning in the study of the stimulating substances working between the gene and the morphological or chemical end-product has been made by Ephrussi, Beadle and their colleagues. "Vermilion" and "Cinnabar" are mutant eye-colours in *Drosophila*; their genes are such as fail to produce the wild-type or normal eye-colour. But if their eye-discs are transplanted into wild-type flies, they develop into wild-type eyes. As the transplants lie free in the body-cavity, a diffusible substance must be involved. But *v* eye into *cn* body gives a wild-type eye, while *cn* eye into *v* body gives a *cn* eye. There must therefore be two substances; the *v* host is deficient in both, but the *cn* host deficient only in one. As *v* may be present with or without *cn* but *cn* is never present alone, a reaction chain is suggested. There is little specificity among species as regards substances governing eye-colour; many other insects contain them. For further details the papers of Ephrussi (2); Ephrussi & Beadle; Beadle & Ephrussi; Harnly & Ephrussi; Ephrussi & Chevais; and Beadle must be referred to.

Chemical analysis of the substances has made some progress. They are heat-stable, not enzymatic, soluble in water and alcohol, but not in ether; dialysable; and certainly not of protein or fatty nature. They are precipitable with phosphotungstic acid and also, like purines, with silver. They thus appear to be organic bases, but so far no pure substances of that kind tried will imitate their action. Nuclein derivatives are inactive. The substances appear to be

destroyed by flavianic, picric and picrolonic acids. For further details the papers of Khouvine, Ephrussi & Harnly; Khouvine & Ephrussi; and Thimann & Beadle must be consulted.

The converse experiments to transplantations, *i.e.*, the injection of lymph from the mutants believed to carry the substances, when tried by Beadle, Clancy & Ephrussi, were perfectly successful, so there is no room for doubt that substances are involved.

Work parallel to that on *Drosophila* has been carried out on *Ephestia* by Kühn, Caspari & Plagge. In this case the testis of wild-type moths produces a substance, known as the A-hormone, which influences a number of characters, the colours of eyes, testes, skin, and brain. Kühn & Plagge have shown that the A-hormone may enter the egg from a heterozygous mother and later affect the larval organs. The A-hormone is not species-specific (Plagge), and preliminary chemical experiments indicate that it is not of protein or fatty nature (Becker).

Mutations, if viable, are but a mild type of abnormality. At the other extreme are the various lethals or semi-lethals. Genes with a complex action, as Grüneberg shows, act in all known cases by setting up a deviating chain of causation. Thus in the new lethal studied by him in rats the first effect is hyperplasia of the rib cartilages, leading to emphysema, from the remote consequences of which the animals eventually die. The studies of Scott on polydactylous monsters in the guinea-pig show that there is an acceleration of growth and differentiation of the limbs, and an inhibition of the rest of the body; the animals die from haemorrhage due to strain on connective tissue where the cervical flexure fails to straighten. Henke and Sinott have provided interesting reviews of physiological genetics, and the important new edition of Goldschmidt's classical book (entirely rewritten) must not be forgotten. In sum, we cannot doubt that the genes largely exercise their functions by way of the production of morphogenetic stimulating substances, or by the inhibition of such production, either through their metabolic origin, or through masking and unmasking (complex formation with proteins).

It has been strikingly demonstrated by transplantation experiments that lethality consists initially in failure of one specific process or death of one specific part. Thus it has long been known that amphibian bastard merogons (*i.e.*, embryos produced from eggs from which the female nucleus has been removed, and which



have been fertilised by the sperm of another species) will not develop beyond an early neurula stage. The origin of their death is a degeneration of the head mesoderm (head organiser). But Hadorn (1) showed that all the other tissues, removed and placed in a normal host, would develop well, and later he showed also that they were capable of perfect self-differentiation when explanted *in vitro* (2). He has now shown (4) that the replacement of head mesoderm in such embryos by normal head mesoderm will lead to normal development, and has studied the effects of the presence of normal tissue in chimaeras made by grafting halves together. Not only does the bastard merogon head mesoderm die; it also fails to exert its normal inductive powers. Similar experiments have been made on mammals and insects; Ephrussi (1) explanted and maintained in normal self-differentiation cartilage, heart cells, etc., from lethal "brachyure" mice, which lack all rump and tail; and Hadorn (3) found that ovaries of "lethal-giant" fruit-flies, normally overwhelmed in the disintegration of the larvae, would live and develop normally if transplanted to normal larvae.

#### EMBRYONIC METABOLISM

For the sea-urchin, Holter & Linderstrøm-Lang have established that peptidase and catalase are enzymes associated with the cytoplasmic ground-substance and not with any granules. Holter & Lindahl find no difference in respiratory rate between animal and vegetal pole halves of blastulae (cf. the work already mentioned on amphibian embryos). The interesting work on the effects of nitro- and halo-substituted compounds on respiration and cell-division by Krahle & Clowes has been continued and amplified. According to Korrr, the unfertilised egg respire through a non-ferrous autoxidisable carrier; upon fertilisation functional connection is established between dehydrogenases and the cyanide-sensitive indophenol oxidase, by the "throwing into gear" of cytochrome. The results of Örström on the sea-urchin egg's ammonia production are rather unexpected. Before fertilisation, whether aërobically or anaërobically, there is a steady ammonia production, but after fertilisation none is formed aërobically, though somewhat more than before anaërobically. Indeed under aërobic conditions, there is now an absorption of ammonia, which may be fifty times as great as the previous ammonia formation. Örström suggests that at fertilisation, large amounts of keto acids are formed. The

change of pH in the blastocoele cavity in later stages has been taken up again with good results by Hirabayashi; it is important in relation to the skeleton-formation of the pluteus. Micromethods have been employed by Hayes for studying the fat metabolism of the sea-urchin egg. His data are more acceptable than his conclusions, since the latter rest on a rather too optimistic weighting of certain points at the scatter-edges.

For the chick embryo there is interesting work by Schønheyder on the formation of coproporphyrin I and haemoglobin during development, and by Imaizumi on its copper and iron. Enzyme activity and its changes have been studied by Galwialo & Goryukhina; Palladin & Rashba; and Goldstein. The synthesis of glycine by the chick embryo has been demonstrated (Patton & Palmer; Patton), and the glycine content of chicks suffering from chondrodystrophy, a lethal condition much affecting cartilage formation, was found to be a good deal below normal. Zorn & Dalton have given us some valuable new data on the chick embryo's blood chemistry; there appear to be considerable oscillations, especially in haemoglobin and cholesterol content. Glucose and uric acid rise more steadily. The anisotropic droplets of cholesterol and cholesterol esters occurring in liver *in situ* and in chorioallantoic grafts, have been studied anew by Dalton (1), who has also again gone into the question of the origin of the glycogenic function of the liver (2). Glycogen first appears in the liver on the seventh day, before insulin can be detected in the blood stream.

Analogous studies of functional differentiation are those of Gersh on the excretory powers of mesonephros and metanephros, both of which are functional at the same time (experiments with glomerular elimination of ferrocyanide and tubular elimination of phenol red). The respiratory rate of the metanephros of the pig foetus, measured by Flexner & Gersh was much greater after the differentiation of the nephrons than before.

Glycolytic processes in the chick embryo during the first week of development have been extensively investigated [see Needham & Nowiński; Needham, Nowiński, Dixon & Cook; Needham & Lehmann (1, 2); Lehmann & Needham]. It is probable that there are two separate routes of carbohydrate breakdown: (i) a nonphosphorylating glycolysis mechanism, very active, and closely bound to the cell-structure, and (ii) a phosphorylating mechanism closely similar to that in muscle, dealing with glycogen and hexosediphos-

phate, but of low activity because deficient in four distinct places: (a) the enzyme esterifying glycogen, (b) the dismutase forming phosphoglycerate and lactate from triosephosphate and pyruvate, (c) lack of adenylypyrophosphate, (d) lack of cozymase. It might thus be said that in early development the phosphorylating glycolysis machinery of the adult is not fully installed. Some sections of it, however, such as the Parnas reaction, are present from the open neural fold stage. Extension of this knowledge to amphibian material would be very desirable.

A notable contribution is that of Bodine & Boell on the effect of dinitrophenol on the respiration of the grasshopper embryo in diapause and while developing. Respiration of diapause embryos, normally low, was stimulated much more than that of developing ones, normally higher. The R.Q. was raised in both cases to nearly unity. The excess respiration, at first thought to be a reversion to carbohydrate oxidation, proved insensitive to iodoacetate, and accountable for by ammonia production as protein breakdown. Further work on cyanide effects in orthopteran development has appeared by Robbie, Boell & Bodine, and on tyrosinase by Bodine, Allen & Boell. Absorption of water by insect eggs during their development has again been shown by a quantitative analysis of those of the capsid bug *Notostira* by Johnson.

Finally we shall mention two reviews, that by Brachet (1) on the partial and complete synthesis of thymonucleic acid by different animals during their development, and that of Irving & Manery on the ionic changes in developing eggs, especially those of fishes.

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GONVILLE AND CAIUS COLLEGE  
CAMBRIDGE, ENGLAND



## GROWTH\*

BY C. B. DAVENPORT, *Carnegie Institution of Washington,  
Cold Spring Harbor, Long Island, New York;*

AND

OTTO RAHN, MARY E. MAVER, H. W. CHALKLEY,  
AND D. M. PACE

Growth, as it has been defined by biologists for the past fifty years, is increase in size. This, combined with differentiation, or the process of specialization of parts, constitutes development. An unfortunate tendency has arisen among non-biologists to use the word growth as synonymous with development, and this has led to the confused state of mind exemplified by Meredith.

### THE GROWTH SUBSTANCE

Hammett (1) has considered the nature of growth in a thoughtful essay, in which he points out that the chemical activity of living substance that leads to growth is the coordinated expression of incremental and developmental factors and functions. Each type of growth expression, *viz.*, initiation, proliferation, differentiation (or chemical specialization), and mass increment must have its own chemical participants.

Since the bodies and skeletal substances of organisms are highly polymerized substances, Mark refers natural growth back to the growth of the chain-like molecule, to polymerism. First, growth centers or germs are formed, due to the concentration of a high amount of activation energy or unsaturation resulting from impact, absorption of photons or other events. Second, any such unsaturated germ may make a growth step if, and when, it collides with a monomeric molecule, uniting with it and retaining its activity. Thus long chains are quickly built up so long as polymerizing substances are available and the process is not broken off by "deactivation" of the molecular complex. As the size of the molecule increases the probability of deactivation by collision decreases. But growth may be finished by annihilation of the active groups at their ends by one of three processes. Under cer-

\* Received October 3, 1938. This review, which surveys the literature to August, 1938, excludes from consideration the growth-promoting hormones in animals and plants.

tain circumstances a colliding monomeric molecule may receive energy from the chain and become transmitted into a new germ, so that as one chain is ended another is started.

The process by which the protein molecule with its polypeptide chain is formed in the cell and by which growth of protein substance in the growing organism takes place has been outlined by Bergmann & Niemann in clear but somewhat speculative fashion. They conclude that the intracellular proteinases are able to degrade proteins through hydrolysis of the peptide bond, even to the formation of amino acids. The same intracellular enzymes are capable of promoting the synthesis of the peptide bond, thereby forming higher order peptides from the above-mentioned products of hydrolysis. Thus the hydrolysis of the extracellular proteins and the synthesis of intracellular proteins are being constantly accomplished under the same general conditions because of small differences in the substrate. It is uncertain whether the proteinases are themselves proteins or contain proteins as essential constituents.

The polysaccharides and proteins in the cell frequently form microscopically visible particles that are closely crowded together, and there assume the form of polyhedra and especially of orthic tetrakaidecahedra (or hexoctahedra). This matter has been especially investigated by Glaser & Child who find that, under compression, visible hexoctahedral units break up into smaller units of the same form, so that eventual molecules of this form are probable. This is in line with the contention of Wrinch (1, 2) that many protein molecules are polyhedra. Marvin has pointed out the law of increase in number as these hexoctahedra are formed in successive layers around the central unit. The total number of units,  $S$ , in any aggregation with  $n$  layers is  $S_n = 4n^3 + 6n^2 + 4n + 1$ . This can be reduced to formal identity with the equation of organic growth,  $\log W = k \log (2t+1) + C$  in which weight is equated against time (Glaser). This is essentially Huxley's equation of relative growth. Hence a better understanding of growth is obtained by appreciating that it follows the law of aggregation of molecules rather than that of intussusception, as held by T. H. Huxley.

The behavior of micellae during growth is described by Sisson for the cotton-fiber wall. Randomly oriented at first they come to have a preferred spiral orientation, varying in different varie-

ties with variation in tensile strength. Castle finds that in like manner *Phycomyces* membrane has a spiral structure.

The mathematical analysis of organic growth, as derived from curves of mass statistics, still occupies a prominent place in the literature of growth. Backman has applied his generalized formula for growth velocities:  $\log h = k_0 + k_1 \log t + k_2 \log^2 t$ , (where  $k_0$  is an initial increment,  $k_1$  is the increment near the maximum, and  $k_2$  the increment further on in the developmental curve;  $t$  is the time at which the increments are taken), to the measurement of growth in weight or linear dimension, in 29 species, including man. He finds in all invertebrates always an embryonic and an infantile growth cycle and in mammals a puberty cycle also. Such a mathematical analysis has been carried even further by Bertalanffy.

Brody considers the relativity of physiologic time and physiologic weight. A given chronologic time-unit has a different physiological time significance in different species and in the same species at different ages. The best method of comparing growth curves is in terms of the eventual or final weight, especially subsequent to the

major inflection. Such curves follow the formula:  $\frac{W}{A} = 1 - e^{-k(t-t^*)}$

where  $A$  is final size;  $W$ , size at maturity;  $k$ , the fractional decline in the velocity of growth with increasing age,  $t$ ;  $t^*$  the position on the age axis where the extrapolated curve of the equation begins at age  $t$  axis: so that  $t - t^*$  is the age counted from  $t^*$ ;  $k$  is the rate of approach to final size,  $A$ . The difference between physiological and astronomical time, as shown by differences (with age) in the rate of growth of tissues that repair wounds, is stressed by du Noüy.

The growth of a piece of a colony of a marine Bryozoon has been shown, by Bronstein, to accord with Brody's formula. And the same formula has been found, by Weinbach, to be applicable to the increase with age of the frequency of alpha brain waves from about three per second at birth to ten in the adult. Other mathematical analyses of growth have been published, especially by Wetzal who has produced a set of equations by which the heat production of growing organisms is expressed directly in terms of the corresponding rate of growth.

#### CHEMICAL AGENTS

As a contribution to the factors of growth the paper of Atlas is important. He measured the oxygen consumption of *Rana* from

fertilization to overgrowth of the operculum. The rate of oxygen consumption increases, without sudden jumps, in proportion to cell number. The rate for the first forty-seven hours is given by the equation,  $Y = 5.31e^{0.038t}$  where  $t$  is the time in hours since fertilization. Growth is accompanied by great absorption of water; respiration does not increase as fast as water content. But two species of frog differing in water content at the same size respire at the same rate.

*Heavy water.*—In confirmation of earlier studies which showed a lag in growth in deuterium oxide, Fox, Cupp & McEwen found that dilute heavy water caused a slowing up of growth of the marine diatom, *Nitzschia bilobata*, but not of *N. closterium*. Hence the effect depends upon the organism studied as well as the concentration of the heavy isotope and probably many other physical and chemical factors. Fischer finds that 25 per cent deuterium oxide inhibits the growth of embryo heart *in vitro*, and completely stops the growth of mouse carcinoma at 100 per cent concentration.

*The role of specific proteins on growth; studies on Obelia.*—Hammett and his associates have continued their experiments on the effect of proteins in solution upon the growth of the hydroid *Obelia*, with results that they consider to be generally applicable. In the initiation of growth Hammett & Porter find xanthine to be active. In proliferation, *l*-hydroxyproline [Hammett (2)], hypoxanthine (Hammett & Steele), and xanthine (Hammett & Porter) play a part. For organization, adenine [Hammett (3)], allantoin (Hammett & Elliott), and xanthine (Hammett & Porter) are important. *l*-Aspartic acid (Hammett & Schlumberger), cytosine (Hammett, Lavine & Lavine), and *l*-hydroxyproline [Hammett (4)] are active in differentiation. Hammett (5) finds these and some other compounds form a natural group by virtue of their relation to pyrrolidone-carboxylic acid. Regression of hydroids is effected by *d*-lysine [Hammett (6)] and allantoin (Hammett & Elliott). A specific dynamic effect is yielded by *l*-proline (Hammett & Collings).

*Sulfur.*—Chapman has found free sulfhydryl groups in all regions of active proliferation in *Obelia*, especially in endoderm. Owen finds that, following necrosis in guinea pig wounds induced by radon, sulfhydryl compounds, such as cysteine, stimulate normal repair. Gregory, Goss & Asmundson, by chemical studies on the

whole carcass of fourteen day old chicks of a large and a small race, respectively, found a consistently greater concentration of glutathione in the larger race.

Gregory & Goss having presented evidence that the concentration of glutathione in new-born rabbits is correlated with body size, Lerner, Gregory & Goss reanalyzed the data by means of the formula for differential growth. The conclusions that each breed has its characteristic glutathione content were confirmed. Toennies reviews our knowledge of the role of the sulfur-containing methionine and calls attention to the need of further investigation. Lwoff reports that certain forms of sulfur are necessary for the growth of certain fungi.

Verne & Verne-Soubiran rendered inactive the embryonic juice of tissue cultures and divided the cultures into two parts; one was given inactive juice, the other the same plus glutathione. The latter showed by far the greater growth.

According to Dufrenoy & Valatx, the roots of seedlings grow on diluted sulphuretted spring waters of the Pyrenees in an accelerated fashion as compared with controls.

*Other chemical agents.*—When *Euplanaria* 16 mm. in length are cut crosswise into eight pieces and placed in proper concentrations of formic or acetic acid (with certain susceptible stocks, propionic and isobutyric acids) the frequency of regeneration of heads on posterior slices is enhanced. If the worm is conditioned by carbon dioxide before sectioning and the sections then placed in propionic acid, the head-regeneration gradient is often almost completely eliminated (Rulon). On the same regenerating species Owen, Weiss & Prince find 1, 2, 5, 6-dibenzanthracene and glutathione to stimulate growth and reproduction; but glutamic acid, glycine, and cysteine are without effect.

#### PHYSICAL AGENTS

*Temperature.*<sup>1</sup>—Different species of marine animals have different maximum temperatures for development. Hoadley & Brill find the maximum for development of *Arbacia* to be 30°C.; the minimum in the annelid *Chaetopterus* is 18°. At temperatures in the lower part of the range the interval between the first and second cleavages is equal to that between the second and third, but at

<sup>1</sup> Cf. also p. 89.

higher temperatures it is greater. Hoadley finds that in frogs' eggs at 30°C. blastopore closure is delayed; microcephaly and modification of the dorsal trunk organs result. However, the morphogenetic processes are affected differentially.

Baccino has reared young rabbits at different environmental temperatures and humidities at various ages. At 34° and 60 per cent humidity growth is better than at 80 per cent humidity; 28° at 75 per cent humidity is optimum for growth from the fifth to the fifteenth day. The optimum temperature for growth varies with age and species.

*Electric potentials.*—Northrop & Burr, in an hypothesis that the pattern of any organism is established by a complex electrodynamic field, refer to the existence of potential gradients over the whole chick or salamander embryo as significant electrical concomitants of the early growth and development of the nervous system. An electrical field has been found to surround the developing embryo up to  $\frac{1}{2}$  mm. from its surface. There is a definite pattern of potential distribution as a whole.

#### INTERMITTENT RETARDATION OF GROWTH

Acceleration of growth after inhibition by cold or other agents has been observed in rats, fish eggs, insects and Cladocera. Buchanan finds that *Amblystoma* eggs kept at 6°C. for three to six days, and then returned to room temperature, have caught up with controls within twenty days. Inhibition of growth by potassium cyanide, followed by return to water, gives a similar acceleration; the cause is still unknown.

Ingle, Wood & Banta find that *Daphnia*, starved from birth until after the beginning of reproductive maturity and thereafter well fed, grows slowly during the period of starvation but markedly increases in body length after being given an abundance of food, even if delayed until the eighteenth instar (when well-fed sisters will have died).

Young mice deprived of solid food on every second, third, fifth or seventh day increased in weight, but less so than the controls (Kopeč & Latyszewski). Those which fasted at seven-day intervals almost equalled the controls in size. When abundantly fed after a fast the test mice came to exceed the controls in weight, since they grew with greater velocity. Comparable results have been obtained by Clarke & Smith in the rat.

Similarly, Fronda, Bañez & Monegas starved incubator chicks intermittently without any serious effect. The chicks which survived long periods of starvation were extremely vigorous, thus indicating a method of developing a superior stock for breeding purposes.

#### GENETIC GROWTH-CONTROLLING FACTORS

Variations in total growth (size) and growth of axial proportions may have a genetic basis. MacArthur & Butler find that in tomato varieties which differ greatly in size the rate of growth and eventual size are anticipated in the ovary by different rates of cell division and varying amounts of cell expansion; these differences in the early anlage are responsible for the relatively enormous absolute differences in size of the mature fruits.

Sprague finds that the hybrids of a large and small strain of maize grow at an intermediate rate, whether the larger strain is used as mother or father. But Jaap finds that in poultry the chicks of two such strains have the growth rate of the more rapidly growing parent.

The internal factors in the rate of growth of poultry are emphasized by Byerly, Helsel & Quenn, as a result of hybridizing bantam Silkies and Rhode Island Reds reciprocally. At hatching, the embryos from Silky eggs, whatever their genetic constitution, were alike in size and the same was true for the embryos from the eggs of Rhode Island Reds. The relative growth rates, after hatching, were initially similar for all four types, but the relative rates of growth dropped after two weeks for Silkies, after four weeks for hybrids derived from Silky eggs, and after six weeks for Rhode Island Reds and hybrids derived from the eggs of Rhode Island Reds. The occurrence of strains of Plymouth Rock chicks varying in their rates of growth has been demonstrated by Schnetzler, beginning with chicks eight weeks old. After two generations of selection the progeny showed a significant difference in rate of growth in weight and adult size.

#### TISSUE GROWTH

*In vivo*.—Parts of the body continue to grow even in adulthood. Basler measured the growth of nails and hair. Both tissues grow intermittently; nails grow fastest between eleven and twelve a.m.,



and at the rate of from 1.5 to  $6.2\mu$  per hour. Hair grows fastest from nine to ten a.m.,  $11\mu$  per hour; during the rest of the day  $55\mu$  altogether.

Small non-toxic doses of sodium fluoride, given periodically to a hydrocephalic infant, caused corresponding deposits in dentine. The average rate of apposition of dentine to the root of the deciduous upper central incisor was found by Schour & Poncher to be  $3.8\mu$  per day, and of enamel to permanent teeth  $2.7\mu$  per day.

*In vitro*.—Mayer, in demonstrating the alternating rapid growth and regression of a fibroblast-tissue culture, finds that only a portion of the cells show immortality.

Venar & Todd kept fresh rat bone growing *in vitro* with vitamin D. The extent and density of mineralization in rachitic bone fragments immersed in a solution of organic calcium were greater than in the controls. Willis implanted into the brains of young host rats the undifferentiated cartilaginous primordia of long bones. The primordia grew at normal rates into the corresponding long bones.

The Creeper fowl is characterized by extremely short legs. David finds that fragments of homozygous Creeper (CpCp) embryos, representing most tissues of the body, when cultured *in vitro* or as chorio-allantoic grafts can survive beyond the ordinary lethal period. The growth retardation characteristic of homozygous Creeper embryos is shown in fragments *in vitro*.

Comparative studies by Hoffman, Goldschmidt & Doljanski of the growth capacity of tissues from embryonic and adult chickens *in vitro* show that colonies of cells derived from the adult organism may exhibit the same growth capacities as those of embryos; but the cells from adult organisms must pass through a long latent period *in vitro* before proliferation sets in.

Of substances that affect the growth of explants, Shipp & Hetherington find no clear effect of allantoin, while Simms & Stillman find a substance in serum that expedites tissue growth (chicken aorta). This substance is not serum albumin, globulin, lipase, or other enzyme. The ultrafiltrate from the serum is highly active in overcoming adult tissue dormancy. No growth is obtained in the absence of the factor, but it is doubtful if it alone is responsible for overcoming the dormancy of adult tissue. (Cf. also "Malignant Growth," p. 99.)

## RELATIVE GROWTH

Huxley & Teissier propose the term "allometry" in place of "heterogony" to denote dissimilar growths of a part from the whole body. The old formula,  $y = bx^k$ , is replaced by  $y = bx^a$ .

In the scorpion, *Buthus*, the growth of each of four pairs of walking legs in total length equalled that of the cephalothorax until the latter was 6.5 mm. Thereafter the legs grew relatively more slowly (Paulian). Davenport (2) cites the fact that the humerus and radius grow at different rates in the infant, though receiving similar growth promoters, as evidence that internal factors (thresholds?) determine the velocity of growth. (Cf. also "Birds," p. 96.)

GROWTH OF BACTERIA<sup>2</sup>

Bacteriologists often speak of growth of bacteria when they mean growth of the culture, *i.e.*, multiplication of bacteria. Their "growth curve" is really a population curve. In most instances, there is no multiplication of bacteria without growth, and factors influencing growth will equally affect multiplication. A difference becomes noticeable, however, in the earliest growth stage (see *infra*) and also when the bacterial crop, the maximal number of cells per unit volume, is concerned because this is a population problem. As an example may be cited the observation of Jensen that in Australian soils, the largest number of bacteria develops between 5° and 15°C. while the rate of carbon dioxide formation by these cells increases rapidly with temperature. This agrees with the findings of Dorn that *Streptococcus lactis* grows most rapidly at 34°, but produces the largest number of cells between 25° and 30°.

*Methods of growth measurement.*—The multiplication of cells is usually computed from agar plate counts or from direct microscopic counts. Shortcuts are sometimes used, among which qualitative turbidity measurements are most common (Faguet; Medwedew & Schelaumowa; Fromageot & Piret; Longsworth; Wright & Kersten).

Relative measurements of the growth rate have been obtained by determining the food consumption or excretion of products in

<sup>2</sup> By Otto Rahn, *Laboratory of Bacteriology, Cornell University, Ithaca, N. Y.* The literature to May, 1938, is reviewed.

short intervals (e.g., hourly oxygen demand). While this is a fair approximation (see however *infra*) no definite relationship exists between maximal number of cells and final amount of fermentation products, e.g., acidity. Orla-Jensen, Eagles *et al.*, and Hutner (1) draw conclusions about the size of bacterial populations from the final titration of cultures of lactic acid bacteria. The limiting factor in acid formation is the pH. Differences in titratable acidity may indicate a buffer effect, a changed acid tolerance of cells, or some other change in bacteria. The same criticism applies to the experiments of Tatum, Peterson & Fred with propionic acid bacteria.

With yeasts, the crops are larger, and are frequently weighed. With molds and Hyphomycetes, dry weight is the most reliable standard. Demeter & Loeweneck found that in a *Penicillium* culture the slightest mechanical disturbance reduced the crop greatly, probably on account of the sinking of spores to the bottom where growth is slower. Young cultures vary more than old ones.

*Growth stages.*—Old cells in a new medium require one or more hours before they multiply at their maximum rate. During this stage of rejuvenation, called the lag phase, bacteria undergo many changes. With three bacterial species, Huntington & Winslow observed an increase in cell volume from 0.3 to 1 c. $\mu$  and in one case even to 2 c. $\mu$ . This volume had decreased again when the maximal growth rate was reached. The maximal rate of metabolism, measured in mg. of carbon dioxide per c. $\mu$  of cells (not per cell), coincided with or closely followed the maximal volume, always occurring before the growth became rapid, and being ten to twenty times as large as that of "resting" cells. Clifton verified this with three other species for carbon dioxide production and oxygen uptake. Wooldridge & Glass observed the same with the dehydrogenases of *Bacterium coli*. Hershey & Bronfenbrenner believe that the increased metabolism of young cells is merely due to their larger size. Rahn, Hegarty & Deuel noticed similar differences with *Streptococcus lactis*. Cells centrifuged from cultures two or three hours old produced about seventy per cent more acid than older cells. Very young cultures which had not recently passed through the rejuvenation stage, did not show this increase.

Hegarty found that the formation of adaptive enzymes is most rapid at this stage of physiological youth. Old cells grown in glu-

cose will not ferment sucrose, but cells one, two, or three hours old attack sucrose within an hour, while cells four or five hours old require two and three hours respectively before they can produce invertase. Similar data were obtained with other sugars.

Moyer found the cataphoretic velocity of *Bacterium coli* to decrease almost immediately after transfer to a new medium. A minimum is reached about one to two hours after transfer, and remains nearly constant until growth becomes slower. This indicates a change of surface during rejuvenation. Watrous obtained similar results with *Bact. typhosum* from broth or agar cultures, but the opposite with cells from his K medium.

The old claim of Gurwitsch that mitosis must be released by mitogenetic rays has been verified by Salkind's observation that bud formation of yeasts is stopped by cancer blood which is able to extinguish all radiation. This ability can be transferred from blood to amino acid solutions without loss of intensity.

Hollaender & Claus concluded that their extensive investigation by biological and physical means had given no indication of the existence of this radiation. However, yeast has never failed Gurwitsch as a detector in more than five thousand experiments. Siebert & Seffert, Barth, and Glaser & Barth obtained good positive results.

From the period of constant growth rate, usually called the logarithmic phase, Hildebrand found that *Erwinia amylovora* required eighty-two minutes, and eleven different species of *Phytophthora* required fifty-five to one hundred and twenty-five minutes for the most rapid doubling of a cell.

Spray gives a summary of his success in cultivating anaërobes in the open, without vacuum or seal, by adding 0.25 per cent of agar to the medium to prevent convection currents and transport of oxygen. Rippel & Lehmann obtained by the same method (0.5 per cent agar) more rapid growth of *Azotobacter*. They explain this by the maintenance of uniform distribution and thereby better food supply, and by increased oxygen adsorption on agar. Virtanen has used semi-solid agar successfully with *Aspergillus*.

A new interpretation of old age is given by Fliegel who believes that the granules developing in old cells of *Bacterium coli* are waste products which cannot leave the cell because the surrounding medium is already saturated with them. Wheeler & Stuart found

that growth of *Bacterium coli* does not cease from lack of food, but from some thermostable, dialyzable substance which also inhibits staphylococci and typhoid bacteria.

*Population growth.*—Recent work on experimental populations of microscopic organisms has been summarized by Johnson (1). Longworth & MacInnes obtained with *Lactobacillus acidophilus* four times as large a population by keeping the pH constant. Birch-Hirschfeld found the maximal number of diphtheria bacteria greatly increased by continuous shaking of the cultures. The crop increased as much as ten times, and the toxin production two to sixteen times.

ZoBell observed that in sea water, larger populations were obtained in smaller containers. This was caused by some bacteria which grew preferentially on the walls of the vessel. Addition of sand greatly increased growth. With additions of more than 10 mg. of peptone per liter, this difference disappeared. Henrici and Stark & McCoy have observed the same phenomenon in lakes. The deciding factor is apparently organic matter, and not temperature, oxygen, or shore contamination.

Different from this periphytic growth is the adsorption of bacteria on soil particles. According to Rubenchik, Roizin & Belianskii, adsorption does not affect the biological properties of bacteria. Only a small percentage can be washed off by prolonged shaking with water. Gunnison & Marshall found that only certain species are removed from cultures with kaolin or similar colloids, and they doubt, for physical reasons, that the phenomenon is true adsorption. It seems to be primarily a property of the species.

The distribution of bacteria between the solid and liquid phases of the soil is probably determined by a combination of the above two principles. The distribution varies greatly with the soil type, according to Nowogrudskii. Usually, the soil solution contains only 0.1 to 1 per cent, in manured soils even 10 per cent, of the total flora of true bacteria, but it contains more actinomycetes than the solid phase. Fehér & Frank developed a formula describing the bacterial population of soils as a function of two variables: temperature and moisture.

*Temperature.*—According to Dorn the optimal temperature of the growth rate of *Streptococcus lactis* is 4° higher than that which gives the greatest number of cells. Foter & Rahn studied growth and fermentation of several streptococci near the minimum tem-

perature. Acid formation proceeded slowly even at  $-0.5^{\circ}$ , but growth ceased near  $+5^{\circ}$ , except for *S. fecalis*. The amount of lactose required for the doubling of one cell remained constant below  $20^{\circ}\text{C}$ . showing uniform efficiency of energy utilization. At higher temperatures, this efficiency decreased.

The optimal temperature of nitrogen fixation by *Azotobacter* in India is  $35^{\circ}$  according to Dhar & Tandon while it is  $28^{\circ}$  in northern countries.

*Other environmental factors.*—While Jennison observed no effect of strong homogeneous magnetic fields upon the growth of micro-organisms, Kimball found that the heterogeneous field of a small horseshoe magnet distinctly retarded the budding of yeast.

Janke found that radon increased the growth rate of yeast by increasing the rate of oxidation, but it decreased anaërobic fermentation.

According to Stapp & Bortels, *Azotobacter* grows better at high barometric pressures.

The literature on oxidation-reduction potentials in bacterial growth has been compiled by Hewitt. Kligler & Guggenheim believe that anaërobic growth is controlled by the redox potential and not by the oxygen tension. Growth is possible in air if the  $E_h$  is low enough. Broh-Kahn & Mirsky interpret their experiments on aerobic and anaërobic growth in the presence of potassium cyanide and various redox catalysts in a very interesting way. Stuart & James claim an effect of sodium chloride on the redox potential of media and of cultures. (Cf. also Longworth & MacInnes.)

#### GROWTH OF PROTOZOA<sup>3</sup>

In *Culture Methods for Invertebrate Animals*, edited by Galtsoff, Lutz, Welch, & Needham many excellent methods are given for culturing *Protozoa*, especially for laboratory purposes, and Pringsheim (1, 2) gives a number of culture methods for various flagellated forms. Most of the investigations made upon unicellular forms are now carried out under well-controlled conditions, which enable the investigator to draw his conclusions from results which are more reliable than they would be otherwise. The following investigators have grown unicellular organisms in well-controlled

<sup>3</sup> By D. M. Pace, *Department of Zoology, Johns Hopkins University*, The literature to August, 1938, is surveyed.

solutions: Loefer (1, 2), *Paramecium bursaria*; Reich (1), an *Amoeba* (*Mayorella palestinensis*); Hetherington, *Glaucoma pyri-formis*; Johnson (2), *Paramecium caudatum*; Phelps, *Glaucoma*; Hutner, *Euglena*; Penn, *Colpoda cucullus*; Pearsall & Loose, *Chlorella vulgaris*; Taylor & Strickland, *Colpoda duodenaria*; Hall & Schoenborn, several euglenoid species; Mast & Pace (1), *Chilomonas paramecium*.

Numerous papers have appeared in the past few years concerning the production of growth substances by the Protozoa. Johnson (2) finds no evidence of the production of any substance which stimulates division or growth in *Paramecium caudatum* but concludes that the rate of division depends upon the density of the food (Bacteria). Phelps suggests that the differences in results obtained in different investigations on the production of growth substances may be due to the different kinds of food used. However, Hetherington suggests that *Glaucoma* may produce an "allelo-catalyst." He obtained a higher division rate when large numbers were added to the culture than when only one or two organisms were added. Mast & Pace (2, 3, 4) present fairly conclusive evidence that a substance, which they call "substance x," is produced by *Chilomonas* which has an accelerating action on growth and they find that very little if any division occurs unless some of this substance is present. They also present evidence supporting the view that as this substance becomes more and more concentrated, it has a retarding action which finally results in an inhibition of growth altogether. The work of Johnson & Hardin on *Paramecium multimicronucleatum* and Taylor & Strickland on *Colpoda* does not support this latter view of Mast & Pace. Reich (2) working with an *Amoeba* in a bacteria-free medium maintains that some substance is produced by the amoebae which augments division and growth.

Leonian finds that plant hormones (from garden peas) show a remarkable stimulation upon the growth of several species of Algae. He also finds that these algae, themselves, produce growth-promoting substances, which can be detected by the stimulating effect of the solution which contained the algae upon the growth of a fungus, *Phytophthora cactorum*. Elliott has tested the effect of several plant hormones on three different types of unicellular organisms but suggests that the action of these hormones is limited to chlorophyll-bearing forms. Hall's results suggest that the same



is true for manganese. As for other inorganic elements that may play an important part, directly or indirectly, in the growth of the cell, Mast & Pace (3) find that silicon acts as a catalyst in the formation of starch by *Chilomonas*. They (5) also find that magnesium and calcium are necessary for cytoplasmic division, although nuclear division may occur when these elements are not present in the external environment.

Several investigators maintain that although algae may be possessed with a mechanism usually associated with photosynthesis, some of them require complex foods. Hutner claims that one or more amino acids and vitamins are needed for growth in *Euglena* and Hall & Schoenborn have been able to confirm these results. Pearsall & Loose used a glucose solution in culturing *Chlorella vulgaris* and have made some very interesting observations on the changes that take place in the metabolic processes during growth. Chalkley (1, 2, 3) gives further evidence in support of his view of the breakdown of the nuclear membrane in ascertaining the distribution of sulphhydryl before, during, and after nuclear division. He finds that sulphhydryl groups are present in the cytoplasm at all times but are concentrated in the nucleus except at metaphase when they have about the same concentration throughout the cell; that is, the nuclear membrane evidently breaks down at this period, allowing the sulphhydryl compounds to diffuse into the cytoplasm.

#### GROWTH OF INFRAMAMMALIAN METAZOA

*Arthropods*.—Teissier shows that the law of Dyer holds in individual arthropods in successive molts, since they increase in geometric proportion.

Measuring forty-two *Daphnia*, some up to seventy-two days, and analyzing the growth-increment curves by the Backman formula, Edlén concludes that their growth consists of two great cycles (embryonal and infantile). These may be widely separated or close together or the infantile may drop out altogether. Extensive growth is associated with a shorter life span, intensive growth with a longer life span. Yet the type of growth has no influence on the size eventually achieved. The type is determined primarily by the environment.

Grasshopper eggs (*Melanoplus*) show developmental block or diapause which diminishes with the age of the mothers, and leads



to variability in the time of hatching. Bodine finds that the oxygen consumption of intact grasshopper eggs is diminished during diapause, while the activity of the enzyme from egg "brei" is constant. The growth of cockroaches supplied with a variety of foods has been studied by McCay.

*Amphibia*.—The effect of age on growth and regeneration in the urodele, *Siredon*, was studied by Syngajewskaja. The fore limb was amputated at the elbow in larvae from 3 to 9 cm. long. The growth of the regenerated limb was highest at first and declined gradually, so that the product of rate of growth and time elapsed since amputation was approximately constant. The curve of regeneration was parabolic. The older the larvae the slower the healing of wounds, mobilization of material for regeneration and differentiation of the fingers.

*Birds*.—In poultry the pectoralis major and the leg bones have a similar  $\alpha$  factor in respect to relative growth in Plymouth Rock and Minorca breeds [Lerner (1)]. But bantams show a different  $\alpha$  value, the leg growth being slower than body growth, suggesting that special growth-retarding factors are present. Applying Schmalhausen's growth constants to data on the growth of Leg-horns and Plymouth Rocks, Lerner & Asmundson find evidence of sex linkage of the growth factor, a higher variance between than within breeds, and a greater difference between sexes the greater the value of  $\alpha$ . Lerner (2) finds that, in poultry,  $\alpha$  for tarsometarsal length in relation to body weight varies in different stages of growth when individual birds are considered, tending to increase from four to twelve weeks and to decrease from twelve to twenty weeks of age.

Comparative studies have been made by Milby & Henderson of the growth rate of Bronze turkeys, White Pekin ducks, and Toulouse geese hatched at the same time and reared on the same ration. The equations used for evaluating growth rates were  $W = Ae^{at}$  and  $W = A - Be^{-at}$ . The growth curves appeared to be divided into three or four periods within each of which the rates were practically constant.

Streich & Swetosarow find that in ducks the hind extremities grow more slowly than the organism as a whole; the fore limb with the same speed. Thus, the anterior appendages come to surpass the posterior in significance, but later this relation is reversed.

In chicks, which are capable of flight at hatching, there is no difference in the development of fore and hind limbs.

#### HUMAN GROWTH

The year has produced a large crop of statistics on child growth taken in masses and individually. The mass data include twelve to fifteen dimensions (Harvard data by Dearborn, Rothney & Shuttleworth; Iowa City white girls by Boynton). Simmons & Todd report, for 950 boys and girls of medium social rank and measured repeatedly, the height and weight and their increments. McCloy gives 149 tables for children four to sixteen years old of triserial correlations, prediction of weight from linear measurements, and "norms" for many dimensions of trunk and appendicular girth and for strength indices.

For babies Davenport & Drager find the curve of weight describable by the exponential curve  $y = 10^{(a+bx+cx^2)}$ ; and Clements finds the growth curve for the second year to be a parabola. Thompson & Gesell and Thompson have measured repeatedly 101 infants during nearly five years and give data on eight dimensions. These do not follow a simple exponential curve. Also, Davenport (3), who has published data on many dimensions of babies for the first year, finds that their various growth curves are due to growth-promoting factors, on the one hand, and growth inhibitors on the other.

Several authors (Hardy & Hoefer; Shuttleworth; Stone & Barker; Richey) demonstrate that girls who grow fast mature earlier than more slowly growing ones. Also, Chenoweth in Cincinnati and Tobler in Switzerland find evidence of a secular increase in the mean height of adolescent children, and Backman (1), referring to Hultkrantz' findings that the mean stature of Swedes is increasing 0.9 mm. per year, points out that measurements of children born at different times must, in mass treatment, be adjusted to this cyclic change. Young and Goldstein & Stanton have made mass studies on the growth of facial dimensions and Goldstein of the head.

Howard maintains that there are three cycles of ossification of bone centers during growth: (a) from fertilization to the end of the first year of postnatal life; (b) a cycle with its peak at the sixth or seventh year; (c) an adolescent spurt. Flory has applied his

studies on growth of ossification and various bodily dimensions to feeble-minded boys.

While it is generally considered that illness affects the growth of infants Hardy finds from repeated measurements of children between eight and twelve years no evidence of a general relation between history of illness and either rate of physical growth during childhood or size at early maturity. Similarly, Gafafer found in thirty thousand elementary school children, divided into those with and those without certain physical defects, no consistent difference in growth rates of the two groups.

Brown finds no significant differences in height and weight in many Utah children under six years of age from urban as compared with rural areas; but as age increases the urban children grow to be slightly taller and heavier.

To aid in judging anatomic age from x-ray examination of bones, Todd *et al.* have published an atlas of the hand taken at six-month intervals. Kelly analyzes sixty-one individual curves, ages six to eighteen years, for anatomic age, and finds that individual stature curves, based on anatomic age, are more varied and complex in form than like curves based on chronological age. The importance of anatomic lag has been stressed by Wallis. This is essentially regression under a new name. Many tables of anthropometric dimensions are given.

Abernethy, comparing the physical age (ossification ratio, standing and sitting heights, weight, chest girth and lung capacity) with the psychological age of 179 boys and 178 girls, measured consecutively two to eight times, and handled mostly as mass statistics, finds a low positive correlation between intelligence and physique; but this result is subject to the criticism that mass statistics of highly variable individuals are inadequate; findings on individuals must be correlated. Simmons & Todd conclude that after two years of age stature is a very competent instrument of prediction over annual intervals—weight somewhat less so. The tallest boys and girls, and also the shortest, consistently maintain their rank.

Since ordinary height-weight-age tables are unsatisfactory standards for judging the bodily condition of children, Dearborn & Rothney give a revision based on the Harvard series of child measurements. This standard is  $\text{weight (kilos)} = 1.73 \text{ chest depth} + 2.11 \text{ chest width} + 0.28 \text{ standing height} + 1.60 \text{ iliac width}$  (all

in cm.)—1184. This equation holds for both sexes, ages fourteen to eighteen.

The chimpanzee's curve of growth, according to Spence & Yerkes, has the same infantile (two to two and a half years), juvenile (three to seven and a half years), and mature periods (seven and a half to eleven and a half years), as the child's. The passage from the positively accelerated juvenile period to the negatively accelerated mature period marks the physiological stage of puberty; but the increase during the prepubertal phase is 25 per cent per year as opposed to the human 10 per cent.

*Technique.*—Davenport (1) proposes a refined technique for measuring the facial features of children and Clark stresses the value of tridimensional graphs. Kornfeld uses a three dimensional coordinate system to plot child age, height and weight, to show from time to time changes in body build. Scammon affords a convenient monogram for computation of fetal age. Hardy & Hoefler use the mean of increments in eight physical traits as anthropometric development score.

#### MALIGNANT GROWTH<sup>4</sup>

Much of the recent advance in the experimental attack on cancer has proceeded from the preparation of pure carcinogenic hydrocarbons and the chemically related hormones. These are being studied not only as etiologic agents, but also with the purpose of learning more concerning the processes of cancerization and malignant growth. In view of the extensive literature in this period (1937–1938), and also the recent excellent article by Cook & Kennaway on "Chemical compounds as carcinogenic agents," we shall survey more particularly the literature dealing with the growth of malignant tissue.

*Metabolism.*—Factors which influence the metabolism of malignant tissue and are correlated with growth are obviously significant in cancer research.

Bischoff & Long (2) point out that caloric restriction *per se*, without dietary deficiency, retards the growth of sarcoma 180. Exogenous protein is necessary for growth. This exogenous protein, as has been shown by Voegtlin, Johnson, Mayer & Thompson,

<sup>4</sup> By Mary E. Maver and Harold W. Chalkley, *National Cancer Institute, U. S. Public Health Service, Washington, D. C.* The literature to July 1, 1938, is reviewed.

must contain certain amino acids—especially essential for the proliferation of the spontaneous mouse carcinoma being an adequate supply of lysine, tryptophane and cystine. Krantz, Musser, Carr & Harne report that the growth of the Walker rat sarcoma 319 was not significantly affected by a diet poor in available sugar capable of glycolysis, nor was it affected by daily injections of sodium fluoride.

Bischoff & Long (1) found the growth of mice unaffected, or slightly inhibited, by thyroxin. The subcutaneous injection of Squibb's anterior lobe extract containing growth hormone produced from 0 to 40 per cent increase in tumor weight over the controls. Lang & Rosenbohm report that long-continued feeding of cholesterol slightly inhibited the growth of the Jensen sarcoma.

Studies *in vitro* of tumor metabolism directly correlated with growth evidently depend on the development of quantitative technics applicable to tissue cultures. This fact emphasizes the value of the study by Victor & Lewis on the metabolism of pure cultures of Walker rat sarcoma 319, covering the respiration, and aerobic and anaerobic glycolytic rates together with the effect of added glucose. They found that the metabolism was that ascribed to fast-growing malignant tissue by Warburg. Long duration of culture had no effect on the metabolism. Added glucose increased the respiratory quotient and decreased the respiratory rate. A report on the growth curves with and without added glucose would have been of interest.

Snellman reported on cultures of Jensen rat sarcoma, in which substitution of fructose or galactose was made for glucose. A decrease of from 40 to 70 per cent in the lactic acid fermentation is reported, but growth was normal.

Litter, Marble & Salter studied the relation of ammonia-sparing by glucose to mitotic activity in normal adult and embryonic liver tissue and in fibroma and sarcoma. They found that the sparing tended to vary directly with the percentage of nuclei in mitosis, and suggest its use as an index of proliferative activity.

*Vitamins.*—Most recent work has been concerned with vitamins E and A. The early experiments of Davidson (1, 2) indicated that mice fed a diet rich in vitamin E were resistant to the carcinogenic factors in tar. Others have found no definite effect on tumor production [cf. Olcott & Mattill; Emerson & Evans; Haddow & Russell]. However, Rowntree, Steinberg, Dorrance & Ciccone

produced transplantable, spindle-cell sarcomas in seventy rats by the oral administration of crude wheat-germ oil; but the nature of the active agent is unknown.

Goerner has devised a centrifugation technic by which the mitochondria of cells can be separated and analyzed. He found that repeated intraperitoneal injections of 1,2,5,6-dibenzanthracene into rabbits markedly reduced the vitamin-A content and increased the total lipid content of the hepatic mitochondria. With small doses of the hydrocarbon the liver regained its power to store vitamin A. Goerner & Goerner later showed that vitamin A is not present in the tumor-cell mitochondria of either the Flexner-Jobling rat carcinoma or the R39 sarcoma in spite of the fact that the hepatic mitochondria of both types of tumor animals have a normal store of the vitamin as well as of total lipid. The significance of these facts in the interpretation of the mechanism of malignant cell division and growth is probably dependent upon further research in nuclear division and cell differentiation.

*Colchicine.*—The problem of the control of nuclear division is of course a basic problem in cancer. One agent known directly and specifically to affect nuclear division is colchicine, which has been chemically studied by Windaus & Schiele. Most authors find that colchicine causes regression of tumor growth, but the findings of Havas & Gál, Brues & Cohen and Brues & Jackson are not in accord with these results. However, Brues & Jackson report that in sarcoma 180 colchicine did not produce complete arrest of mitosis as it did in regenerating liver in rats.

*Hormones.*—Studies on the effect of sex hormones on tumor growth have been stimulated by the chemical relationship of estrin to the 1,2,5,6-dibenzanthracene type of carcinogenic agent. Earlier workers have shown that ovarian hormones influence both the genesis and development of malignancies. Lacassagne, continuing previous studies on the production of mammary carcinomas by estrone, has found that spindle-cell sarcomas appear in mice after prolonged treatment (injection) with estrogenic hormones. Champy was able to produce ovarian adenomas in guinea pigs (animals which are quite resistant to cancerization) by the injection of folliculin and by the oral administration of testicular lipoids for several months. Bonser, Strickland & Connal induced five mammary carcinomata in 32 non-breeding female mice of a tumor-resistant strain by subcutaneous injection of estrone benzo-

ate, estrone and prolactin. No tumors appeared in 41 untreated mice. Gilmour showed that the follicular hormone did not stimulate the growth of a transplanted mouse tumor. When the ovarian hormones have been supplemented with carcinogenic agents their effect upon the cancerous transformation is more marked in mice. Perry & Ginzton report the induction of neoplasms in a colony of female mice, one-half of which were painted with 1,2,5,6-dibenzanthracene and theelin. The incidence of carcinoma of the skin appeared to be related chronologically to the development of breast carcinoma, and breast carcinoma was "causally and chronologically related to carcinoma of the uterus." Nitta was able to accelerate the growth of the Flexner-Jobling rat carcinoma and the Fujinawa rat sarcoma by injections twice daily of the hormone of corpus luteum.

Unlike the potent carcinogenic hydrocarbons, the sex hormones appear to promote continuously the growth of the tumor which they have helped to induce. Loeb, Burns, Suntzeff & Moskop in a careful study of the hormones and their relation to tumors state that,

If we take into consideration the various data which have now been discussed, it appears most probable that specific growth stimuli, acting over a long period of time, ultimately change the cell equilibrium in such a way that certain substances inducing cell proliferation are propagated in an autokatalytic manner. As far as is known at present, all the causes of cancer directly or indirectly stimulate growth processes.

This subject is also reviewed by Gardner and by Geschickter & Astwood. Earlier work has shown that extirpation or irradiation of the pituitary gland retards tumor growth. More recently Zondek has produced dwarfism in rats by the administration of follicular hormone which he has shown prevents the growth hormone of the anterior pituitary from entering the blood stream. A transplantable, benzpyrene-induced tumor grew as well in the dwarf as in the normal control rats. Zondek points out that mere blocking of the pituitary might not have the same action as the entire removal or destruction of the gland. Since the question of secondary disturbances of nutrition and metabolism arises in hypophysectomized animals, Ball & Samuels studied the influence of nutritional factors on Walker 256 tumor, and found that a balanced food intake at a low level did not eliminate the difference in tumor size between the hypophysectomized and normal rats.



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CARNEGIE INSTITUTION OF WASHINGTON,  
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## TEMPERATURE REGULATION\*

BY ALAN C. BURTON

*The Eldridge Reeves Johnson Foundation for Medical Physics  
University of Pennsylvania, Philadelphia, Pennsylvania*

Of recent comprehensive reviews of temperature regulation mention should be made of that of the general field by Bazett(1) in 1927 and that of the physical factors concerned by Deighton (2) in 1933. This annual review covers papers published between January 1937 and August 1938. Reference to earlier papers is made only where no later papers on the same topic or by the same authors have been found.

The constancy of body temperature depends upon the maintenance of a balance between the heat production and the heat loss of the animal body. The former depends on the metabolic activity of the tissues alone, the latter on both physiological and physical factors. As far as heat flow from the internal tissues to the skin surface is concerned, we deal with physiological variables; beyond that point, in the heat flow through the clothing to the surrounding air, with physical factors which are largely beyond immediate physiological control. It is the realization and separation of the two types of factors, physiological and physical, in heat regulation that has been the major advance in this field of recent years. The study of the regulation of body temperature consists in finding out how a change in any term or terms of the equation of heat balance is compensated by a physiological change of some other terms. It is convenient to consider heat loss by evaporation in a separate term from that by other routes of loss, for though the same physical factors are involved, its physiological control may be in part distinct.

### CONSTANCY OF BODY TEMPERATURE AND DEVELOPMENT OF REGULATION

The function of temperature regulation is not completely developed at birth, nor is that of the adult free from considerable limitations. The progress of its development in children, as shown by an increasing constancy in the diurnal cycle has been studied by Kleitman *et al.* (3). They find a gradual increase in the regularity

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of the diurnal cycle and decrease in its range of temperature during the first year, leading to a definite establishment in the second year. Here, however, the range is still greater than that of adults and the minimum temperature averages below 36° C. Van der Bogert & Moravee (4) have also studied this in more than 750 children. Of those which showed a temperature above 99° F. during the daily routine, 43 per cent were seven to eight years old, 33 per cent were from nine to thirteen years, and only 8 per cent were older. We must, therefore, conclude that development continues for several years. In the hedgehog full development takes 31 days [Eisentraut (5)], in rats some twenty days [Gulick (6)].

The diurnal rhythm of temperature in apes has been studied by Shcherbakova (7). The whole field of diurnal rhythms in biology has been reviewed by Welsh (8). There is still no evidence that the rhythm of temperature does not merely reflect the cycle of activity.

A very complete study of the fluctuation of temperature in the menstrual cycle of women had been made by Harvey & Crockett (9). This is now supplemented by a study of the coincident fluctuations of basal metabolism by Rubinstein (10). These amount to ten to twenty per cent while in males the fluctuation is only three per cent. The low temperature phase, which is eight to twenty-one days post-menstrual, is the period of least homeostasis.

Cases where human temperature regulation is completely functional but operates at an abnormally low (11) or high (12, 13) level are recorded by Reimann. No explanation is at present offered.

#### THE HEAT-REGULATING CENTER

It is now almost universally accepted as established that a "physiological center" situated in the hypothalamic region is essential to the regulation of body temperature. The only recent opposition is that of Thauer and his coworkers in Frankfurt. They claim that when section of the brain stem of rabbits is made just behind the hypothalamus "a high degree" of thermal regulation persists in chronic preparations (14). This they found also after complete extirpation of the midbrain (15) and, in previous work, after lower transections of the spinal cord (16). They conclude that the brain center is not essential and that the peripheral

nervous system must be capable of assuming to some extent the functions of temperature regulation. The use of rabbits, animals in which the major role in physical regulation is played by the ears, the innervation of which was unaffected in some of the operations, makes these conclusions open to doubt. With guinea pigs, rats and mice clear evidence of the restoration of heat regulation was not obtained (16). Issekutz and coworkers (17, 18) found that cervical transection of the cord in dogs does not abolish but merely decreases the resistance to cold. They emphasize that not until the last nervous pathway from the brain center to the periphery has been intercepted, including the vagi, inferior cervical and stellate ganglia, does the animal become truly poikilothermic. The thyroid was shown to play a role in the restoration of partial regulation in operated animals. Hermann, Morin & Galy (19) also found that removal of the entire lumbar sacral cord has little effect on the resistance of dogs to cold (10° C.). The question of sympathetic fibers leaving the cord very early and proceeding via the stellate ganglion was raised.

The existence of many subsidiary mechanisms that play a rôle in the complete scheme of temperature regulation, including the adrenal and thyroid glands and possibly a control of metabolism by metabolic products, need not minimize the importance of the brain center in the regulation which is mediated by the nervous system. The clinical finding, cited in support of his views by Thauer, of tumors of the hypothalamus (20, 21), without disturbances of temperature regulation may indicate that the "physiological center" has a strictly localized anatomical existence. More and more exact localization has proceeded. Frazier and coworkers (22) had placed the center in the mesial hypothalamic nucleus of the cat and in the substantia grisea in man, close to the floor of the third ventricle. Laterally placed lesions did not abolish regulation. Later work has indicated an anatomical separation in the cat of centers controlling heat loss and heat production. Lesions in the anterior part of the nucleus impair the resistance to heat without serious effect on the resistance to cold [Ranson *et al.* (23)]. They found the same differentiation in the monkey (24). The most recent paper is that of Keller (25) who, by transections properly placed, was able to obtain cats and dogs showing loss of the heat-production mechanism, yet with retention of heat-loss control. Experiments of Ectors *et al.* (26) and of Crouch & Elliott (27)

using electrical stimulation of the hypothalamic centers have shown differences in autonomic and motor localization. Magoun *et al.* (28) used low voltage high frequency currents with Horsley-Clarke electrodes in the cat's brain, feeling that this gives a local thermal rather than electrical stimulus. They found that the effective region is concentrated in the medial portion of the caudal part of the ventral telencephalon. An acceleration of respiratory rate, panting and sweating were used as indices of a response of the heat-loss mechanism.

Morgan (29) has observed changes in the cells of the nuclei of the hypothalamus after fever induced by typhoid and bronchisepticus toxin injection. Sixty percent of the cells of the nucleus tubero-mammillaris showed chromatolytic change, while other nuclei were unaffected. He has some evidence that the thyroid is involved in the fever mechanism.

Indirect evidence of hypothalamic control abounds. Rosenthal & Friedländer (30) found that antipyretics are effective in much smaller doses when injected into the region of the tuber cinereum than when given subcutaneously. Sympathin and epinephrine are liberated on electrical stimulation of the region [Magoun *et al.* (31)]. Disturbances of heat regulation in chronic encephalitis (32) and in dementia praecox (33) are attributed to lesions of the hypothalamus.

It is well known that other parts of the brain may influence temperature regulation. This is reviewed by Kennard (34). According to Pinkston & Rioch (35) cortical areas 4 and 6 are important in vasomotor control. Erlich (36) reports six cases of prolonged fever without complications following removal of the cerebellum.

#### TEMPERATURE RECEPTORS AND SENSATION

A new field has been developed by Hardy & Oppel with their studies of the sensitivity of the peripheral heat and cold receptors using refined physical measurements of the stimulus, here infrared radiation focused on the skin. They have shown first that radiations of different wave lengths are effective in producing temperature sensation only in proportion to their absorption by the skin (37). Since the non-penetrating radiation is the most effective, the depth of the end-organs must be unimportant. They have determined the minimum temperature change of a large area of skin that can

elicit a temperature sensation (38) to be a rise of only  $0.003^{\circ}\text{C}$ . on the forehead, at a rate of  $0.001^{\circ}\text{C}$ . per sec. This rise is produced by a radiation intensity of  $0.00015\text{ cal. per sq. cm. per sec.}$  falling on the skin, a quantity only one nine-millionth of the normal hourly radiation loss of the subject. The first requirement of a heat regulatory mechanism, namely a temperature sensitive device, is, therefore, provided with what must be a more than adequate sensitivity. As the area irradiated increases, the threshold intensity required decreases, indicating a spatial integration by the nervous system of the impulses from receptors over a wide area (39). By having an area of skin radiate through a conical reflector to a block of solid carbon dioxide instead of to air, the stimulation of cold spots has similarly been studied (40). The number of cold end-organs is greater, but the spatial summation is poorer, and the threshold more than five times as great as for heat end-organs.

Geblewicz has been working in the same field using a similar technique but studying temporal rather than spatial summation. He has compared the intensity of stimuli of varying short durations with that of a constant stimulus that gives rise to the same sensation (41), finding that sensation reaches its plateau in from three to twenty seconds. A curious feature is that the maximum effect is reached sooner, the less intense the stimulus, the opposite from what would be expected from behavior of other receptors. On the other hand, using two successive stimuli separated by a variable interval (42), he found that the thermal receptor follows the same law as the visual receptor.

O'Connor (43) has continued his series of papers which claim to show that the response of cold end-organs is correlated with the oxygen consumption of the skin. The statistical validity of his conclusions seems open to question. The existence of a chemical factor in the stimulation of temperature receptors, suggested by the discovery of the carbon dioxide effect by Liljestrand & Magnus (44), has been confirmed by Gollwitzer-Meier (45). In a fresh-water bath  $35.2^{\circ}\text{C}$ . is the neutral temperature, but in baths saturated with carbon dioxide  $33.0^{\circ}\text{C}$ . may appear neutral and in these baths the rectal temperature steadily falls until shivering occurs. Gellhorn *et al.* (46) have reported a curious decrease in the errors of localization of warmth stimulation when tactile heat stimulation is used instead of non-tactile (radiation) stimulation.

even though no conscious touch sensation was produced. Woll (47) has found that repeated applications of cold ( $10^{\circ}\text{C.}$ ) to the skin diminishes selectively the thermal discrimination of cold.

The diffuse distribution of temperature end-organs with other receptors in the skin has been a stumbling block to the use of electrophysiological methods in their study. The description by Noble & Schmidt (48) of a localized organ of temperature sense in the facial pits of snakes offers hope of investigation in cold-blooded animals. These snakes when blindfolded can detect their prey by temperature discrimination sensitive to  $0.2^{\circ}\text{C.}$  Most inconveniently for the physiologist, the group of snakes concerned is that of the large pythons. Extremely sensitive temperature organs may be found in elasmobranch fishes, according to an interesting paper by Sand (49) who has recorded the action currents from the ampullae of Lorenzini. A rise of temperature of as little as  $0.1^{\circ}\text{C.}$  causes a marked inhibition of their spontaneous activity, while a fall accelerates it. After an adaptation time of five to ten seconds a final frequency is reached, which however shows the usual temperature variation, *i.e.*, it is greater the higher the temperature. These temperature receptors, then, if such they be, appear to have a great sensitivity to changes of temperature, acting in this case like "cold" receptors, while the sensitivity to steady temperatures is slight and in the opposite sense. The lateral line receptors of the fish show a very much smaller temperature sensitivity. These had been discussed as temperature receptors by Hoagland (50) and by Rubin (51). We should perhaps be cautious in accepting these ampullary end-organs as normal temperature receptors until the function of the long, thin-walled, jelly filled tubes that connect them to the surface of the head has been elucidated.

#### PHYSICAL REGULATION

*Heat loss by radiation and convection.*—1936 was marked by the emergence of a new method for measurement of heat exchanges of man with his environment [Winslow, Herrington & Gagge (52, 53)]. Called by the authors "partitional calorimetry", the method does not make direct measurements of heat, but, by measuring all the factors that by known physical laws determine radiation and convection from the body, can give by calculation the heat exchanges. With determinations of respiratory metabolism to give

heat production, of body temperature to give heat storage or debt, and of evaporation losses by weighing the subject, the items for the heat balance are complete. A feature of the method is that by adjustment of the temperature of the air and of the radiating walls of the enclosure, radiation and convection losses of the subject may be independently varied. Great service has been done in the formulation and establishing of the physical laws used in the calculations of heat exchanges. The authors introduce a new scale of "operative temperatures" (54) to express the thermal demands of the environment on the organism. This scale, by taking account of radiation, is designed to improve upon the older "effective temperature" scale of Yaglou. It is unlikely that the general public will ever use any of these scientific scales, but a new popular term "humature", a scale obtained by averaging the dry bulb temperature and the percentage humidity, made its appearance in the heat wave of 1938 and may be adopted by the man in the street.

The use of the Hardy radiometer (55) in the respiration calorimeter at the Russell Sage Institute by Du Bois & Hardy has permitted a separation of radiation and convection in their work, since total heat is measured by calorimetry and radiation loss is estimated separately by the radiometer. The result of the work from the two laboratories has been the presentation of charts showing the partition of radiation, convection and evaporation losses of the human body over a large range of environmental conditions of temperature, humidity and air movement. A sample partition given by Hardy & Du Bois (56) is that, of the total heat loss, radiation accounts for 58 per cent, convection 15 per cent and vaporization 27 per cent. The partition is so dependent upon particular conditions that the results cannot be discussed in any detail here. The references (52 to 63) are merely listed. Convection loss approaches zero when the external temperature reaches 34.5° C., while vaporization which at 23° C. is only 17 per cent, at 35° C. must account for the total heat loss (60).

The finding that there is no significant difference in the thermal properties of white and negro skin by Hardy & Muschenheim (59) has been confirmed by Laurens & Foster (64).

After these extensive and thorough researches, it is difficult to see how our knowledge of this part of the subject can be much further advanced, and we must expect this chapter soon to be closed.

*The circulation in regulation.*—Studies of the temperature and

heat loss of the body surface yield their full physiological meaning only when they are interpreted in terms of the peripheral circulation and its physiological adjustments to temperature. The use, in recent years, of the "thermal conductance" of the tissues has made this interpretation possible. To be found in principle in the work of LeFèvre (65), this concept was developed by Burton (66) and has now been adopted by Hardy & Du Bois (61) and by Winslow *et al.* (58). The conductance is found from the equation:

$$\text{Conductance} = \frac{\text{Rate of total heat loss from surface}}{(\text{Area of surface})(\text{Rectal temperature} - \text{skin temperature})}$$

Measurement of the heat loss by calorimetry, or calculation from the physical laws governing loss from the surface of the body to the air, enables the conductance to be calculated. It depends primarily upon the peripheral circulation, though its value is, of course, affected by fat deposits. Winslow *et al.* (54) have found that the conductance varies from vasodilation and constriction between forty and ten kilocalories per square meter per degree centigrade per hour, figures agreeing remarkably well with those of Burton & Bazett (67). Hardy (61), however, has found a much smaller range, the minimum being reached, in the naked subject, at an external temperature of 28° C., so that below this temperature physical regulation would be ineffective. Clothing extends the range down to 25° C., but even this seems remarkably high for the lower limit of physical regulation. The calculation of the conductance is invalidated when the body temperature is changing, and in this case, errors of unknown amount may result which may account for the disagreement. The nature of the relation between the effective thermal conductivity and the rate of blood flow in a limb is at present only assumed. Winslow *et al.* (54) have suggested the equation:

$$\text{Total conductance} = f + (c/d)$$

where  $f$  is the flow of blood,  $c$  is the specific thermal conductivity of the tissues without blood flow, and  $d$  is the "depth of the gradient." It seems doubtful whether we can consider the two pathways of heat as strictly "in parallel," as implied in this formula, but some very similar relation must hold.

Sheard and coworkers (68) and Grant & Pearson (69) have



shown that the greatest vasomotor changes are in the extremities, which are the most effective dissipators of heat. The changes are greatest in the fingers, less in the hand, and still less in the forearm. The reactions in the toes are even greater than those of the fingers according to Doupe *et al.* (70). Burton (unpublished data) found that in the fingers the blood flow has the enormous range of from 1 to over 80 cc. per min. per 100 cc. of tissue. Similar conclusions as to the relative roles of the extremities in heat regulation had been reached by Uprus, Gaylor & Carmichael (71) and recently by Freeman & Nickerson (72).

Hicks and coworkers, using an oscillometric technique to record the pulsation of the radial artery (73, 74, 75) have studied the effect of environmental temperature on the skin circulation of Australian aborigines, who live in a rigorous climate without clothing. Comparison with the behavior of whites at the same temperatures shows that the ability of the natives to withstand greater cold is due to an earlier and more complete constriction.

Shunting of the blood occurs in vasomotor reactions. Bazett *et al.* (76) have obtained evidence from pulse-wave velocities that the large arterial trunks dilate on exposure of the subject to cold, and according to Friedlander *et al.* (77) the changes in flow in the muscles is often of opposite sign from that of the skin.

The studies of Freeman & Zeller (78) on the blood flow in the sympathectomized paw of a dog show that even here the flow varies considerably with the local temperature of the tissues, indicating a "metabolic" control of blood flow. How far this operates in the normal animal as a heat regulating mechanism is uncertain.

If vasoconstriction is ineffective in preventing the chilling of the peripheral tissues, a reactive hyperemia sets in as a protective reaction. This has been shown to be an axon reflex by Wybauw (79), by Hermann *et al.* (80) and by Springorum (81).

*Insensible perspiration and sweating.*—The statement that loss of heat by insensible perspiration amounts to 25 per cent of the total heat loss has been regarded merely as a rough rule. Newburgh and coworkers (82, 83) found however, that when loss of weight is followed in twenty-four hour periods in subjects who pursue the normal routines of life, excluding periods of intense muscular activity, this percentage is remarkably constant. The average was 24.4 per cent, with a variation from 23.8 to 25.2 per cent in different

subjects. Using the average of several twenty-four hour periods the metabolism may be calculated from the insensible loss to within five per cent. The age group of four to fifteen years has been studied by Ginandes & Topper (84). They find a correlation coefficient of  $0.94 \pm .0045$  between insensible loss and metabolism. In infants the percentage is markedly less, according to Levine *et al.* (85) and Gordon & Kelly (86). Levine *et al.* have found that when temperature is raised or humidity increased, the increase in evaporation loss is accompanied by a decrease in the urinary output of water.

In contrast, Gasnier & Mayer have been studying the variations in the weight loss of rabbits in short periods, and have derived a "coefficient of irregularity." They have found that the total loss and its irregularity may be differently affected by drugs (87 to 90). Effects of starvation (91) and of water ingestion (92) have been studied. The weight losses of the cat have been similarly studied (93), before and after sympathectomy (94). They conclude that control of insensible loss shows both cholinergic and adrenergic factors (95) and in a general discussion (96) formulate ways of studying the precision, sensitivity and rapidity of the regulation.

A considerable advance in clarifying the relation of the physical and physiological factors that control evaporation from the body has been made by Gagge (97). By comparing the evaporation from the body with that which would occur from a surface of water at the skin temperature under the same conditions (a quantity that may be calculated from the physical conditions), a "percentage wetness" of the skin may be derived. This remains at about ten per cent for the range of insensible loss, rising to one hundred per cent after sweating begins. The effect of humidity has been studied over a wide range (98).

Winslow *et al.* (54) and Hardy & Du Bois (60) agree that sweating begins at a critical average skin temperature of from 34° to 35° C. In water baths, however, 35° C. is a neutral temperature (45), and sweating occurs only at considerably higher bath temperatures. At an average skin temperature of 35° C. in air, some areas have a much higher temperature than this, while in water baths the normal differences of temperature between different areas of the skin are eliminated.

Using an iodine-starch reaction as indicator, List & Peet (99) have made beautiful photographs of the distribution of the sweat glands over the body and of the differences to be found with lesions of the sympathetic (100). They show differences in distribution of emotionally and thermally induced sweating, the latter being much less localized.

#### CHEMICAL REGULATION, SHIVERING AND THERMAL MUSCULAR TONE

The question whether there exists a true "chemical regulation" apart from extra metabolism of muscular origin evoked by cold is still a matter of debate. It has long been realized that there may be changes in the "tone" of muscles which give rise to extra heat without any sign of the tremor of shivering in these muscles. The work of Burton & Bronk (101) has provided an objective measure of this "thermal muscular tone." When the body temperature of cats is lowered below a critical level, there is a rise in the activity of the muscles shown by their action currents, due to an uncoordinated asynchronous twitching of the individual muscle units, which is simultaneous with the first signs of a rise in metabolism. Only in a later stage does this type of activity pass into the coordinated firing of units that shows itself in the gross tremor of shivering. The onus seems, therefore, thrown upon those who study the increase of metabolism to cold "without shivering," to show that it is not to be attributed to an unperceived rise of muscular tone, if they are to prove that they deal with a true extra-muscular chemical regulation.

The important rôle played by the adrenals in cold resistance cannot, of course, be denied. Recent work in this field is that of Horvath and coworkers (102). On exposure to 4° C. the metabolism of normal rats increased 170 per cent over the basal value, while in unilaterally adrenalectomized animals the increase was 139 per cent, and in doubly adrenalectomized 81 per cent.

Two methods of distinguishing between muscular and true extra-muscular chemical regulation have been advanced by the workers in France. Chevillard *et al.* (103) have found that when the oxygen tension is lowered, mice lose the power of thermoregulation against cold. Giaja (104) has found however that the basal metabolism is unaffected by lowering oxygen tension until lethal

effects are produced, and that the power of shivering is unaffected. The effect, therefore, would seem to be selectively upon true chemical regulation. Cord section does not abolish this regulation (104). Gellhorn (105) has found that the fall of body temperature in rats under low oxygen tension is accelerated by the addition of 3 per cent carbon dioxide. Since the latter is known to improve the conditions of oxygenation at low tensions, the loss of heat production cannot be ascribed directly to low oxygenation of the tissues.

The second differentiating feature according to Kayser & Dell (106, 107) is that true chemical regulation utilizes preferentially an oxidation of fat, while in shivering the R. Q. rises, indicating oxidation of carbohydrate. Support has been given by Dontcheff and coworkers (108, 109) who have found a parallelism between the increased metabolism and a fall of blood lipids. They conclude that the "hormones of cold," epinephrine and sympathin, catalyze the oxidation of fat. Bazett *et al.* (110), however, finding low R. Q.'s in cool baths, without shivering in the subject, attribute them to acid-base shifts of the blood consequent to lowered temperature.

Jung, Doupe & Carmichael (111) have studied the relative effectiveness of peripheral and sensory stimulation in the production of shivering in man. With peripheral stimulation, a response was easily elicited before the rectal temperature had fallen more than  $0.01^{\circ}$  C. and sometimes even with a rise of rectal temperature. They do not deny, however, that fall of blood temperature alone may be an adequate stimulus. It may be noted that Gollwitzer-Meier (45) found that the subjects eventually shivered after the rectal temperature had fallen  $0.3^{\circ}$  to  $0.5^{\circ}$  C. in carbon dioxide baths where peripheral cold sensation was lacking.

Jung and coworkers (111) by the use of patients with various lesions established that the nervous pathway for shivering involves the cerebellum and the anterolateral columns, and not the pyramidal tracts or the cerebral hemispheres, confirming Uprus, Gaylor & Carmichael (112). The presence of muscle afferents is necessary for the appearance of the clonus-like movements of shivering (112), a fact also suggested by Burton & Bronk (101).

A curious fall of body temperature of rats in "oxygen poisoning," produced by exposure to oxygen under four atmospheres pressure, is described by Campbell (113). If this fall is prevented by high external temperatures, the animals die. We must regard

this then as an instance where the temperature regulating function is subordinated to a more vital protective mechanism.

### FEVER

A discussion of the recent literature on fever would occupy too much space for its inclusion here. The subject may, therefore, conveniently be omitted as pertaining to pathology rather than to physiology. One event of fundamental interest must, however, be mentioned. Barbour and his coworkers (114, 115) had shown in a series of researches that changes of body temperature were accompanied by a disturbance of the normal osmotic relations of the blood serum, blood concentration accompanying a rise of body temperature. The theory that anhydremia was a primary factor in fever production had gained wide acceptance. This conclusion is now challenged by Rogers (116), who has found in man that the rise of blood density precedes the rise of body temperature, persists only a short time and that the density may return to the normal level while the temperature continues to rise. At the crisis of the fever and afterwards there may be blood dilution. He concludes that the blood concentration is not an essential factor in the fever but is a part of the vasoconstriction of the anaphylactoid reaction. Support is found in the work of Black (117) and of Anderson (118) in which the changes in insensible loss in pneumonia have been followed. Gibson & Kopp (119) have followed changes of blood volume in fever, while Palitz (120) has found that the increase in red cells in artificial fever is due to a prolonged splenic contraction.

Of great interest, if foreign protein contamination can be ruled out, is the report by Lumière & Meyer (121) that the injection of inert colloidal solutions in serum of animal charcoal, of rouge and of Troy white produce a fever reaction, which appears to depend upon the physical state of the injected substances. This would suggest a surface action on the cells of the heat regulating center.

Hemingway has been studying the respiratory responses in artificial fever of dogs (122, 123, 124). Panting starts abruptly at a relatively constant level of skin temperature, which seems to be independent of the rate of rise of skin temperature or the environmental temperature. With a rise of respiratory rate up to 350 per min. the volume of tidal air decreases some 25 per cent, indicating

the existence of a mechanism by which maximum evaporation is secured with a minimized extra loss of carbon dioxide and resulting alkalosis. Cats, in contrast to dogs, never pant unless the rectal temperature is raised, according to Kleyntjens (125), peripheral stimulation being ineffective.

#### SLOW ADAPTATIONS TO CLIMATE AND EXTREME CONDITIONS

There has been of recent years a new interest in those slow adaptations of the organism to external temperature which by common experience are known to take place, for example, in the shift of the human "comfort zone" of temperature between winter and summer. Consideration of these must certainly be included in any review of heat-regulation. Because of these adaptations experiments made in summer may produce results that differ significantly from those of winter experiments [as emphasized by Mills & Ogle (126)].

Sunderman, Scott & Bazett (127) find a 15 to 40 per cent increase in human blood volume in summer over winter. In a winter experiment in which the subjects remained in a hot room, increases of 7 per cent the first day, and 29 per cent after five days (28 per cent in four days in a repeated hot period) were found, representing a total increase of blood volume of about one liter. Cardiovascular changes were also observed (128). The same group of workers has repeated the experiments in summer, but results are not yet published. The increase of blood volume must be connected with a marked increase in the peripheral vascular bed. In the summer experiment referred to there was evidence of a progressive increase in the maximum rate of blood flow in the fingers for five days in the hot room [Burton (129)]. Summer and winter differences in the curves of tissue thermal conductivity in baths of different temperatures were noted by Burton & Bazett (67).

On the other hand McLain & Montgomery (130) have found dehydration and blood concentration in workmen exposed to high temperatures, while Adolph & Dill (131) found a remarkably constant water content of the body. Dill (132) has reviewed the older findings on blood volume in his recent book. We may understand the disagreements, if we conclude that acclimatization to moderately high temperatures is accompanied by an increase of blood volume, while in extremely hot conditions or those of heavy work,

where sweating must bear the whole responsibility for heat loss, blood concentration results. Under these extreme conditions, increase of blood volume to allow an increased maximum skin circulation would in any case be ineffective in increasing heat loss.

In 1937 the Harvard desert expedition made by Dill and co-workers resulted in the gathering of much data regarding adaptation of man to extreme conditions. Here the body must accept heat from the environment in amounts even greater than the metabolic heat [Adolph (133)]. The adaptations are of two types, first, an increased ability and readiness to sweat (131), and, secondly, a salt economy consisting in a progressive decrease of chloride concentration in the sweat (134), which may fall in the course of a week to half its normal value. This salt economy had previously been studied by Daly & Dill (135). Mezincescu (136) found a much smaller decrease in concentration. Adolph & Dill also made studies of the water balance of a burro (donkey) (131), which like the dog [Adolph (137)] makes a very rapid adjustment by water ingestion. Comparative studies of the guinea pig, rabbit, goat and cat subjected to high temperatures have been made by Schuhecker (138).

There is no doubt that endocrine factors are involved in these slow adaptations to climate. Bazett (personal communication) thinks that the pituitary may be concerned in the changes of urine output occurring in the adaptation period. In the adaptation to cold climate in rats, we have much evidence of the rôle of the thyroid. After a sojourn in the cold, the basal metabolism of rats is markedly increased, even if measured at neutral temperatures (139, 140). This effect is seen even in fish (141). The changes are attributed to the thyroid, activity of which is increased at low temperatures [Woitewich (142)], and which is more active in winter than in summer (143, 144). Chevillard *et al.* (145) have found that the weight of the liver increases in mice kept in the cold, and Simpson (146) has reported an effect upon the islets of Langerhans. The emptying time of the human stomach markedly decreases [Sleeth & Van Liere (147)], which may explain the increased appetite in cold weather.

#### AIR CONDITIONING

The recent development in both the scientific and the commercial aspects of air conditioning and public education in its desira-



bility has and will stimulate interest in the branch of physiology to which this review is devoted. Surveys of the subject are given by Yaglou (148, 149), by Winslow (150), and by Drinker (151). Many papers of physiological interest appear in the pages of the Journals of the Institute of Heating and Ventilating Engineers, of the American Society of Heating and Ventilating Engineers and in the Journal of Industrial Hygiene. It is disappointing to find in a report on the incidence of sickness in one thousand employees working in air-conditioned rooms that no significant difference was found from those in other rooms [Bristol (152)]. Similar negative results were obtained in experiments in the Metropolitan Life Insurance Company Building by McConnell (153). We must conclude with Drinker (151) that at present air-conditioning cannot be justified on the grounds of improved health, however justified it may be on other grounds of desirability.

#### CONCLUSION

A reviewer should summarize in conclusion the most significant advances in the field of recent years, and give some account of the present state of the problem to indicate the gaps in our knowledge that remain unfilled.

The last two years of research have yielded advances in the measurement of the partition of heat losses in the animal body, and in the separation of radiation from convection losses. By the interpretation of heat losses and skin temperature in terms of the thermal conductivity of the superficial tissues, and of losses by evaporation in the terms of the physical equations governing evaporation, a clear separation of the physical from the physiological factors has been achieved. A new field has been opened up by the careful study of the sensitivity of thermal receptors and of the integration of their activity by the nervous system. Here we still have to depend upon subjective sensation as an index. Steady progress has been made in the anatomical location of the essential center in the brain and some information as to the nervous pathways involved, especially in the shivering response to cold, has been forthcoming. A new interest in the slower adaptations of organisms to environmental temperature conditions has revealed suprisingly great physiological differences between the summer and winter animal, and points to the importance of endocrine factors in heat regulation.

In the category of unfinished business we must include the clear demonstration of the relative importance of increased muscular and extra-muscular metabolism in the regulation against cold, and the elucidation of the mechanism of such extra-muscular increases. In some respects our descriptive knowledge of the heat regulating system is approaching completion, but this throws into contrast our complete ignorance of the fundamental physico-chemical mechanisms of its functioning. We do not know why the activity of a thermal receptor and of the cells of the heat regulating center are uniquely sensitive to changes of temperature, how a fall of temperature can excite a cold receptor or increase the activity of the center responsible for cold resistance, nor what is the change in the cells of the center selectively effected by a fever producing agent or by an antipyretic. In these matters we have not reached even the stage of tentative hypothesis. There is no doubt that the subject of heat regulation must continue to appear in the *Annual Review of Physiology* for many years to come.

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THE ELDRIDGE REEVES JOHNSON FOUNDATION FOR MEDICAL PHYSICS  
UNIVERSITY OF PENNSYLVANIA  
PHILADELPHIA, PENNSYLVANIA



## ENERGY METABOLISM\*

BY JOHN R. MURLIN

*Department of Vital Economics, University of Rochester,  
Rochester, N. Y.*

Feeling the need of a term which would designate energy metabolism above basal, the writer several years ago suggested the term "supermetabolism"<sup>1</sup> to include the effects of all the known factors which produce a rate of heat production higher than basal in normal subjects. Basal itself, below and above normal levels, should be called hypo- and hypermetabolism in accordance with the general usage of these prefixes. These distinctions will be observed in this review.

### NORMAL STANDARDS

We are fortunate in having within the current year continuations of several important series of papers calculated to clarify the limitations of normal heat production in man. Berkson & Boothby continue their series on the basal metabolism data collected at the Mayo Clinic in a study devoted to intra- and interindividual variability. The intra-individual variability is greater for females than for males while the interindividual variability from mean metabolism is greater for males. Both forms of variability can be satisfactorily represented by the Gaussian or "normal" distribution curve. In successive intradaily observations there is a trend exhibiting first a decrease then an increase. The authors assemble the data on their large series of 639 males and 828 females by age into two groups: (a) those under twenty years, and (b) those twenty years and over. The standard deviation for males under twenty years was 3.14 kilocal. per sq. m. per hr. or 6.5 per cent of the mean; for those twenty years and over, 2.58 kilocal. or 6.7 per cent of the mean. For females the standard deviation in the group under twenty was 2.98 kilocal. per sq. m. per hr. or 6.8 per cent of the mean, and for those twenty and over 2.42 kilocal. or 6.9 per cent of the mean. This is total interindividual variability, meaning

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<sup>1</sup> By analogy with *basement* and *superstructure* of a building.

the comparison of different individuals of the same sex and age. Comprised in this, however, is the variability of single individuals, each about his own mean. For this intra-individual variability the authors found ten females and twenty-three males with a sufficient number of determinations for statistical treatment of the data, limited for the sake of homogeneity to fifteen consecutive observations within a period of three months. For males the standard deviation intra-individually was 1.33 kilocal. per sq. m. per hr. or 3.5 per cent of the mean, and for females 1.61 kilocal. per sq. m. per hr. or 4.7 per cent of the mean. The intra-individual variation proved to be only about one-half as great for males and two-thirds as great for females as the interindividual variation.

The series of recalculations of heat production by Poulton & Adams which has run for several years in *Guy's Hospital Reports* have reached a climax in Professor Poulton's (1) address as president of the physiology section of the British Association for the Advancement of Science. In this he has summarized many of the points brought out in earlier papers. A fundamental error alleged by Poulton in the work of Benedict's laboratory is that when the difference between indirect and direct heat is plotted against the respiratory quotient a systematic error is found such that with low R.Q.'s the indirect heat is too high and with high R.Q.'s it is too low. The same is true to a lesser degree of DuBois' results. When values for carbon dioxide and oxygen are plotted against heat (from the same sources) the carbon dioxide points much more nearly coincide with theoretical heat values based on analyses of human fat and Lusk's heat value for carbohydrate. In the printed paper, however, the author makes a significant correction from the paper as read, for he finds in many of DuBois' results that the eliminated heat has been overcorrected for changes in body temperature (rectal). These overcorrected points are the very ones which give too wide a scattering of the oxygen thermal quotients. The author, however, strongly favors the theory of a constant combustion ratio (heat/carbon dioxide). He states that DuBois' results, studied with reference to all variations in the R.Q., are compatible with this theory, and those between 0.7 and 1.0 are compatible also with the theory of variable carbon dioxide thermal quotients. In any case, carbon dioxide alone need be measured.<sup>2</sup>

<sup>2</sup> Cf. King, J. T. Jr., *Basal Metabolism*, (Williams and Wilkins 1924).

Poulton (2) has supplied some new evidence of the trustworthiness of carbon dioxide as a measure of the B.M.R.<sup>3</sup> obtained principally on untrained subjects by the method of Krogh & Lindhard in successive five minute periods. Analyses were made for carbon dioxide only. For comparison one would have liked to see oxygen figures also.

In their last contribution (available to the writer), based on recalculation of the work of others, Adams & Poulton have considered the metabolism of children. They find that the relation<sup>4</sup>  $\log W/\log H$  provides a measure of "general nutrition" in childhood, confirming Brody as to the inflexions of the curve. They advocate prediction of the B.M.R. from height for subjects below 41 inches (corresponding to 5 to 6 years), and from weight for subjects above this height.

More impressive evidence to the same effect, as regards older girls at least, comes from the statistical study of Talbot, Wilson & Worcester. Besides a new series recently reported by the same authors (1935) they include the results of basal measurements in fifteen other series reported by different observers in different parts of the world, twelve of them in the U.S.A. The authors were frankly most concerned to select a standard for practical clinical use, *i.e.*, one which would give the "truest clinical picture for persons with abnormal and pathologic conditions." At the conclusion of a very involved statistical treatment that only a mathematician trained in statistics can understand, they conclude that the "total calories for the weight gave the closest fit of any method used for predicting calories for the groups of girls studied" and that there is a certain amount of evidence that the same would hold for boys. They call attention, however, to the necessity of allowing for variations with latitude, age and "different eras of life." Speed of growth in particular they found related to surplus metabolism. More recently, Talbot has supplied two tables which predict the total basal calories per 24 hours for both boys and girls, one based on weight and the other on height. He notes that since the figures for basal heat production given in the height standard were obtained from children of normal weight for height the standard can be regarded as a height standard for "expected weight."

<sup>3</sup> B.M.R. = basal metabolic rate.

<sup>4</sup>  $W$  = weight;  $H$  = height.

The progress report of Lewis, Kinsman & Iliff in their very imposing longitudinal study of the metabolism of children by the cot-chamber method is very satisfying to the physiologist. It covers about four years of work and gives evidence of great care in planning and execution. Fifty-two boys and 41 girls between two and twelve years, all normal children and representing all social strata in the city of Denver were studied as age progressed. Adequate and understandable statistics were applied to the raw data. Simple diagrams which facilitate rapid reading were used. They found that calories per hour referred to weight, the same referred to surface area (DuBois; Boothby & Sandiford) and calories per hour per square meter referred to age gave the most concordant results (lowest coefficients of variability). There were only slight differences amongst these coefficients.

Three new studies on boys and girls, girls and young women, boys and young men continue the series from the University of Naples. Miani-Calabrese collected data on 481 boys and 369 girls between the ages of six and seventeen and analyzed them statistically. The B.M.R. fell with increasing age, weight and height but not regularly from year to year or from one height or weight interval to the next. In general the fall was more marked for girls than for boys, and the B.M.R. of boys generally was higher for any given age, weight or height. In company with Perrelli, Miani-Calabrese found that the average B.M.R. of 136 females decreased from 42.87 kilocal. per sq. m. per hr. at twelve to thirteen years of age to 37.22 kilocal. at seventeen to eighteen years. From this time on the B.M.R. was constant to age twenty to twenty-one. Lafratta obtained B.M.R. measurements on 213 pupils of the Military College of Naples. The group fourteen to fifteen years of age had a rate of 45.03 kilocal. per sq. m. per hr. while the group nineteen to twenty years of age could show only 43.05 kilocal. Except for the group sixteen to seventeen years of age, of which the B.M.R. was lower than for the next biennium, the decline was continuous.

A notable study of the changes with age in basal metabolism (oxygen only) in 100 men over forty years of age is reported by Lewis (1). There were twenty men in each of five decades. The B.M.R. declined at a nearly uniform rate from 40 years on, the dependence upon age being given in kilocal. per sq. m. per hr. by the expression  $39.138 - 0.0678 \times \text{age}$ . In each decade the regression

was 0.664 kilocal. per sq. m. per hr., indicating a declining curve less steep than those of Harris & Benedict, of DuBois, as modified by Boothby & Sandiford (1929), and of the more recent one of Boothby, Berkson & Dunn. For the evenly distributed group between 40 and 89 years the mean metabolism was 34.814 kilocal., the standard deviation 2.813 (8.1 per cent of the mean) and the standard error 0.282. The author has reported (2) also on the cardiac index (liters output of blood per sq. m. per min.) of the same subjects. In general the decline in this index parallels the decline of oxygen consumption.

Confirming an earlier deduction from observations on full-fed baby chicks, Kleiber has recently found that fasting baby chicks show an increase in metabolic rate with increasing age. This increase was 0.2 to 1 per cent per day per unit of body weight, over the extrapolated rate of metabolism at birth. Based on the author's unit ( $W^{3/4}$ ) it was 2 per cent per day; and on surface area, 3 per cent per day. The study was limited to the age range five to twenty days. Barott, Fritz, Pringle & Titus found the B.M.R. of Rhode Island Red chicks fasted for 66 hours was highest at fifteen days of age. It decreased rapidly from that point and became constant at one hundred days. With rats of advanced age Benedict & Sherman found a higher total basal metabolism than for rats of the same weight in middle age. In advanced age the B.M.R. tended to become stabilized at about 100 kilocal. per kg. of body weight per day, or 700 kilocal. per square meter of body surface ( $9.1 \times W^{2/3}$ ), as noted for old women by Benedict & Meyer in 1932. Black & Murlin, using a new automatic calorimeter, found the total metabolism of several groups of rats, fed in different ways, to decline up to about six to seven months of age, after which it rose. Busse studied by Warburg's method the respiratory metabolism of kidney tissue and diaphragm muscle in prematurely-born, newborn and young guinea pigs. The  $Q_0$  values for the diaphragm muscle of prematurely-born pigs were less, in the proportion of 3.6:5.0, than those of pigs born at term; during the first three weeks after birth the  $Q_0$  varied but little. With kidney tissue the corresponding ratio was 8.3:10.6. Since the metabolism of the two tissues ran different courses, it may be concluded that the gradual rise with age in the basal metabolism of the organism as a whole is a resultant of the specific metabolisms of the various tissues, and not the resultant of generalized hormone or nervous regulation.

Do young leucocytes produced under stress require more oxygen than mature cells? Ponder & MacLeod have found that cells of a fourth successive peritoneal exudate in rabbits have a respiration rate 50 per cent greater than those of the first exudate.

F. G. Benedict, retiring director of the Nutrition Laboratory of the Carnegie Institution of Washington, presents his valedictory discourse on basal metabolism in a monograph of 215 pages entitled "Vital Energetics." It is a comparative survey of the metabolism data on all the warm-blooded animal species, including man, ever studied in his laboratory. Adult animals only are considered. Eighteen species of mammals, including man, and seven species of domestic and several of wild birds are comprised in the intraspecific and interspecific comparisons on warm-blooded types. The species range in weight from the eight-gram dwarf mouse to the four-thousand-kilogram elephant. A critique of the surface area concept is given including a consideration of the metabolism at 16° as well as at 28° or thermal neutrality. The final comparison is that between warm-blooded and cold-blooded animals, the latter both at low body temperature and at 37° C.

The author lays great stress upon the importance of turning "from a consideration of heat loss to a consideration of heat production." He advocates comparing the metabolism of animals of the same or different species that have the same body weight, as a partial way out of the difficulties of comparison. Marked sex differences come out in the data pertaining to certain human races and certain animal species (cattle, pigs, goats). The monograph concludes with a consideration of the factors that may contribute to metabolic differences within or between species, such as body structure, body composition, body covering, cell (body) temperature, enzymes, brain weight and blood. The author believes that progress will be made in future metabolic studies

by discarding all thoughts of a uniformity in heat production, and by seeking to associate differences in metabolic intensity, or vital energetics, with differences in configuration and body make-up and with differences in morphology, chemistry, and above all, distribution of blood.

This monograph represents a prodigious amount of work; it is unified by standard techniques; the data are adequate for the most part for statistical treatment; and, the determination of the director of all this work and author of its recital to observe the

most scrupulous care to sift results honestly and not to be swayed by preconception is manifest on every page.

The only new formula for surface area encountered in the literature of the year is that of Stevenson for Chinese subjects. It is based on 100 linear (DuBois) formula determinations embracing a wide range of normal variability of Chinese body sizes and shapes,

$$A = 0.0061H + 0.0128W - 0.1529 \pm 0.0254$$

$A$  is surface area in square meters;  $H$ , stature in cm; and  $W$ , weight in kg.

#### THE QUESTION OF A RACIAL FACTOR

Benedict (2) sets forth the extreme position in favor of this factor. He believes the studies on South Indian women showing a basal rate 17 per cent below the Harris-Benedict prediction and those on several tribes of Indians, wild Chinese and Mayas of Yucatan all showing a metabolism higher than that of Caucasians reveal genuine racial differences.

Goewie & Radsma express the opinion that there might be a question of social status concerned in some of the measurements on tropical groups. They present results on 25 native male (?) servants and 33 native male (?) students in Batavia (Netherlands Indies) to show that this may be the case. The former gave an average  $0.6 \pm 1.005$  per cent above the Harris-Benedict standard, while the latter gave  $4.5 \pm 0.445$  per cent below. They admit, however, that the average is "somewhat arbitrary," as some values were excluded from the data on the servants without a sharp criterion of selection. Ages also in the servant group were somewhat uncertain, and the R.Q. measurements probably not quite so reliable. The authors do not regard the averages given as final proof of the influence of social level, but maintain that the question has not had adequate attention. Agreement with this viewpoint is expressed by Ellis, Wilson & Roy who report on the basal metabolism of 62 (mostly Hindu) boys from six to sixteen years of age. The average results were lower in varying degrees compared with the Aub & DuBois, Boothby & Sandiford or Nakagawa standards, but somewhat higher than the Benedict (modified) standard. Taking all standards into consideration, the authors conclude that the metabolism is on the low side, but considering the fact that all of the boys were underweight and on a low-protein diet, they believe



it would be unjustifiable to favor the hypothesis of a racial factor being the cause. Oliviero whose observations embrace seven races in Singapore declares race has no significant influence on basal metabolism. Miller & Benedict with their 258 cases, representing at least five races besides several mixed groups in Hawaii, could not make out an uncontaminated racial factor. Against the evidence of Benedict (2) that Chinese women have a basal metabolism considerably lower than that of Caucasian women is the contribution of Siddall & Kwok on Southern Chinese women. Single observations with the Sanborn-Benedict apparatus on subjects while still in bed were made on 100 women (students) of an average age of 21.45 years (range 15 to 36). The average weight was 43.5 kg. as contrasted with 54.5 kg. for American women of the same average age. The average B.M.R. was -6.4 per cent calculated according to the original Aub-DuBois standard. This is not lower than the level of many groups of American women living in the Southern states. The trend of the most recent literature, therefore, is away from the idea of a racial factor.

The only new work from the Southern states is that of Nalbandov, Heller, Krause & Purdy who present a large number of observations on the basal metabolism of Oklahoma men and children. In agreement with the report of Coons (1931) on the basal metabolism of Oklahoma women, the authors find that of men to average 5.63 per cent below the Aub & DuBois standard. Oklahoma children agree with northern standards up to the age of seven for boys and eleven for girls, beyond which they become lower.

#### INTERNAL FACTORS AND CORRELATIONS

Terroine and associates in previous years have shown, as they believe, that a close relationship exists between the excretion of creatinine and the intensity of energy metabolism. The idea has already been applied by employing the creatinine coefficient as a criterion of nutrition, as a gauge of obesity and even as a substitute for basal measurements. However, Wang did not find in children so close a correlation of preformed creatinine with basal metabolism as with body weight, albeit the former was high. The ratio, creatinine coefficient: B.M.R., rose steadily with weight. Terroine and his collaborators now present data on the rat which relate creatine excretion to thermogenesis in response to cold. (See

p. 156.) This would seem to imply that so long as the demands for heat are not unusual the breakdown of phosphocreatine during rest and its normal dehydration to creatinine measure the energy production; but when the demands for heat are increased up to the onset of shivering the excretion of creatine commences. Creatinuria, however, certainly has other causes only remotely related to thermogenesis to cold. For example, Lohmann has followed up the observation of Brentano on the relation of glycogen depletion (creatinuria) to carbohydrate metabolism. Rabbits were the subjects and creatinuria was produced in various ways (hunger, urethane, thyroxin, glycine, etc.). In every instance but one there was a diminished utilization of sugar, as proved by dynamic effects and the R.Q., when creatinuria existed. The latter condition therefore may be accepted as a sign not only of glycogen destruction but of diminished combustion of carbohydrate.

Ashworth & Cowgill (1) have studied the relationship between endogenous nitrogen excretion and basal heat production in the rat. The data of Brody, Procter & Ashworth (1934) and of Smuts (1935) seemed to prove quite convincingly a close correlation between these two quantities for several species of mammals. Ashworth (1935) first questioned this relationship for growing animals; he and Cowgill have now tried to account for variations in the quantity kilocal. per mg. of urinary nitrogen by appeal to body composition. Data were accumulated on twenty rats varying in initial weight from 46 to 350 gm. They were kept on nitrogen-free diets for four days after which a collection period of five days was used for endogenous nitrogen and, following this, the B.M.R. was determined in a four to six hour period. With consideration of the standard errors it was found that the best correlations were obtained between kilocalories and/or urinary nitrogen on the one hand and body nitrogen and fat-free dry weight on the other. The best correlation was not independent of weight. Plotting the ratios of endogenous urinary nitrogen to basal calories per day against body weight, an approximately straight line was obtained only in the range 50 to 100 gm. body weight. Outside these limits, the ratio varied from less than 1.0 to nearly 3.0. It therefore follows that in the rat a definite proportionality between these metabolic factors is of very limited application. Synephius finds the ratio of endogenous nitrogen to basal energy quite variable from species to species.

Does the shift from carbohydrate oxidation to fat oxidation and vice versa, in obedience to Rubner's law of isodynamic replacement, affect the pH of the blood sufficiently to change the rate of oxygen utilization? If so, might we not say that the shift comes about by the effect of pH on the oxidative catalysts? With these questions in mind Kane & O'Connor studied the influence of the pH of blood plasma on oxygen consumption in relation to the law of isodynamic equivalence. Rabbits were the experimental animals and the pH of the blood was altered in different ways, (a) by changing the depth of artificial respiration (under anaesthesia), (b) altering the carbon dioxide content of the inspired air, (c) by intravenous injections of hydrochloric acid up to 50 cc. of 0.1 *N* in 0.6 per cent saline, (d) by intravenous injection of sodium bicarbonate. The oxygen absorptions were determined at 36, 31 and 25° C. At the two higher temperatures it was found that a fall of pH below about 7.6 caused a decline in oxygen consumption of approximately 5 per cent for a fall in pH of 0.1. For the isodynamic replacement of fat by carbohydrate a fall in pH of at least 0.08 or, more probably, 0.11–0.2, would be necessary. Changes of this order may occur normally. At 25° variations in pH between 7.2 and 8.3 did not influence oxygen consumption.

#### HYPOMETABOLISM

Not many physiological factors are known as yet clearly to depress the basal metabolism below the normal level. Aside from undernutrition (see Chambers) and sleep, only a few endocrine deficiencies or antagonisms can be mentioned. Rubenstein (2) correlated the course of body temperature with B.M.R. readings (Benedict-Roth apparatus) throughout the menstrual cycle of fifteen young women. The average B.M.R. was 10.5 per cent below the normal Boothby-Sandiford modification of the Aub-DuBois standard. The low point in each curve came near the midpoint of the cycle, thirteenth day, coinciding with the most highly cornified (ovulative) vaginal smear. The high point in the B.M.R. occurred about five days premenstrual. The basal metabolism, however, was independent of body temperature [Rubenstein (2)]. Subjects living on a constant diet, sleeping in air-conditioned rooms and having their basal metabolism taken six days of every week for fourteen months showed an average seasonal variation of only 3.5 per cent (low point in the autumn), according to a preliminary report of

Lockwood & Griffith. The menstrual variation in two women subjects was 6 per cent, with the low point at the eighth day of the period.

An inquiry, similar to that conducted on normal rats, was instituted by Ashworth & Cowgill (2) for hypophysectomized rats. It was found, in agreement with previous investigators, that this operation reduces the B.M.R. to about 60 per cent of normal, but does not appreciably affect the endogenous urinary nitrogen. Consequently the daily ratio, urinary nitrogen/kilocalories, comes out much higher than for normal animals.

Sherwood, Wilson & Boneta have shown that amniotin does not cause a reduction of the B.M.R. in ovariectomized rats after the thyroid also is removed. The reduction due to thyroidectomy was of the same order as had been previously reported (1933) when amniotin had been given to otherwise normal ovariectomized rats. But Sherwood later found that amniotin apparently has the power to neutralize the prolonged effect of thyroxin. Thyroidectomized rats presented a hypometabolism of 30 per cent; desiccated thyroid substance raised it 79 per cent in five days with return to the base line in twenty-four days. Amniotin administered near the peak of hypermetabolism caused return in twelve days.

Anterior pituitary extracts can quite definitely decrease the rate of oxidation of glucose, to judge by experiments of Meyer, Wade & Cori on rats, at the same time increasing by a corresponding amount the deposition of glycogen in liver and muscles.

#### HYPERMETABOLISM

*Thyroid.*—Boothby, Berkson & Plummer have compared with their normal standards the B.M.R.'s of pathological subjects. Those with diseases supposedly not affecting the B.M.R. showed a mean 2 per cent higher than that of normal subjects and a larger variability ( $-18$  to  $+22$  per cent). Considering the histograms of exophthalmic goiter, adenomatous goiter and spontaneous myxoedema on the same axes as the normal distribution, it was found that almost the entire distribution of the B.M.R. for the first group was "skew" in the right or positive direction and almost the entire distribution for the last-named group was skew to the left, or negative direction. Referring back to their statistical work on normals the authors place double emphasis on the fact that B.M.R. always must be treated as a variable quantity and con-

sequently no one lowest value has a prerogative to be considered the correct basal over any other determination made under proper conditions.

Hoff, Gentzen & Klemm have reported five subjects, who, in connection with encephalitis, developed a thyrotoxicosis or hypermetabolism or exophthalmos as isolated symptoms. The hypermetabolism fell promptly in those subjects treated with the hypothalamic narcotic "prominal" (Merck). Reduction was obtained also in other forms of thyrotoxicosis. Prominal is a barbiturate which like others of the group keeps down the hypermetabolism induced by thyroxin. The authors report several experiments of this kind on guinea pigs. Prominal did not affect the histological structure of the thyroid as changed by thyrotropic hormone. The same was true of liver glycogen. The activating effect of thyrotropic hormone upon the thyroid was maintained in prominal narcosis even when the hypermetabolism was checked. Treatment of thyrotoxicosis with the narcotic must therefore be looked upon as a symptomatic treatment, which holds down the metabolism during its action without really affecting thyroid function at all.

On the moot question of the participation of higher centers in the stimulating effect of thyroxin on energy metabolism, Mansfield & Tyukody performed cross-circulation experiments in which the head of one dog was provided with blood from another, and found that the complete narcotization of the head (nerves and spinal cord intact) with luminal produced no reduction of the metabolism previously raised by thyroxin. Injection of large doses of epinephrine into curarized, decapitated dogs did lower the metabolism and it is to this hormone the authors attribute previous apparently successful demonstrations (Izzekuts) of reduced metabolism when the spinal cord was cut. Epinephrine used to maintain the blood pressure was shown to have this effect if administered in amounts such as 10  $\mu$ g. per kg. per min.

In the early part of her monograph Büllman describes some procedures for the determination of very small quantities of iodine in the blood. The iodine combined with protein amounts to about 5  $\mu$ g. per cent. While the chemical composition of the protein-bound iodine fraction was not known, it was assumed that in the main thyroxin was concerned, since a relation to the intensity of metabolism could be demonstrated. When the B.M.R. was plotted

as percentage of normal against the concentration of protein-bound iodine in  $\mu\text{g. per cent}$ , a band was obtained whose mean value was given by the formula: Percentage of basal  $-a = b \log$  iodine concentration ( $\mu\text{g. per cent}$ ). The agreement with values found was excellent.

Cohen & Gerard fed rats on dry thyroid tissue for sixteen to nineteen days, until they had lost four to ten gm. in weight, at which time the brain was removed and minced to study its respiratory activity. The  $Q_{O_2}$  was initially 30 per cent higher than that of normal brain suspension. The  $Q_{O_2}$  of hyperthyroid brain was approximately four times as high as that of normal when substrates were added which are involved in carbohydrate oxidation and glycolysis. With paraphenylenediamine the increase over normal was 33 per cent. The absolute concentration of various enzyme systems was greater in hyperthyroid brains than in normal. Shibata found the oxygen consumption of anterior pituitary tissue increased by previous injection or feeding of thyroid tissue to rabbits, that of posterior pituitary was decreased, as was also that of adrenal cortex and medulla.

*Anterior pituitary (thyrotropic and gonadotropic factors).*—There have been numerous reports that the thyrotropic hormone acts to increase respiratory metabolism *in vitro*. After investigating the mechanism of the calorogenic action of this hormone on tissue metabolism, reported by Paal (1934), Canzanelli & Rapport found the latter's fundamental observations to be correct as regards thyroid tissue but not as regards liver. Incubated with either tissue and with one of the three substances, thyroxin, thyroglobulin or diiodotyrosine, thyrotropin did not activate any preformed stimulant or produce one from any of the three substances named; its action therefore would seem to be directly on the thyroid cells. Mahaux found that daily injections of this hormone increased the metabolism of guinea pigs for a few days, then ceased, although still producing exophthalmia. O'Donovan noted that a substance in pituitary extracts, differentiated from the thyrotropic factor, caused in the rabbit a transient rise in oxygen consumption up to 40 per cent.

Sellei & Mayer were not able to demonstrate any measurable effect of pure gonadotropic hormone ("Luteoantin," Richter) on the respiratory metabolism of the kidney of infantile white mice. They had previously demonstrated an increased metabolism in the

kidney of tumor-bearing animals, apparently due to the tumor itself.

*Anterior pituitary (Growth hormone (?) in pregnancy).*—Johnston *et al.* give results on the basal metabolism of a primipara through the last two months of pregnancy and two months *post partum*. Data are given also on the complete nitrogen balance, the accumulated nitrogen and sulfur gain and the total calories ingested. The increase in basal heat produced from the eighth to tenth lunar months of pregnancy amounted to 300 kilocal. per day. The percentage increase in kilocal. per sq. m. per hr. was 14 per cent (from -3 per cent to +11 per cent of normal). A marked parallelism is recorded between the retentions of nitrogen and sulfur and the basal heat, which suggests a relationship to total protoplasmic mass. By assuming that the basal calories from protein would be represented by one twenty-fourth of the nitrogen output in the day's urine, a relative fall in the calories from protein and a relative rise in calories from fat were found. This fact, together with evidence of increased pituitary activity in pregnancy, suggests that the increase in basal metabolism and nitrogen retention are governed to some extent by the growth hormone of the pituitary, as shown by Gaebler. The nitrogen curve and basal calories, however, did not run parallel in the lactation period.

Hanna agrees with Hughes (1934) in finding a gradual rise in the B.M.R. of normal pregnancy after the fifth lunar month and hypermetabolism amounting to as much as 33 per cent in the ninth lunar month, although "generally it was only slightly elevated above the usual normal limits." Effkemann & Borgard on the contrary found the B.M.R., on the average, increased by 8 per cent in the fifth lunar month, 12 per cent in the sixth, 17 per cent in the eighth, 18.5 per cent in the ninth and 19.1 per cent (range 8 to 38) in the tenth. They ascribe this result principally to hyperactivity of the entire endocrine system and particularly of the thyroid.

*Anterior pituitary (prolactin (?) in lactation).*—Riddle *et al.* showed that prolactin increased the basal metabolism of pigeons independently of the thyroid. Brody, Riggs, Kaufman & Herring have studied the respiratory metabolism of rats during gestation and lactation. The metabolism in gestation was not increased beyond the normal proportion,  $W^{0.73}$ , for the weight increase. The metabolism of lactation, on the other hand, was enormously in-



creased beyond this proportion. This is contrary to findings reported in the literature. Since others have found that the gestating rat does not consume more food than the non-gestating, it follows that energy must be saved in other ways. The failure previously to find an increased metabolism in lactation may have been due to the fact that lactation nearly stops in the postabsorptive condition. Brody has also compared the gross energetic efficiency, *i.e.*, relation to total digestible nutrients, of milk production in goats with that in cows and rats. The smaller the animal, the more milk energy it produced per unit of body weight. However, the net energetic efficiencies in large and small animals tended to approach each other. It is of special interest that the basal metabolism and production of milk energy tended to change at the same relative rates with changing body weight. More directly Graham, Houchin, Peterson & Turner gave attention to the energy cost of milk production in goats. Analysis of arterial and venous blood from mammary vessels for oxygen and carbon dioxide, and corrected as to carbon dioxide for possible formation of urea, gave R.Q.'s which averaged 1.36 indicating a formation of fat from sugar. Estimations of the blood flow and of the energy value of the milk produced a gross efficiency of approximately 90 per cent; consequently the cost of producing 1000 kilocal. in milk would be, roughly, 100 kilocal.

*Ovary.*—The respiratory metabolism of the liver, kidney and anterior lobe of the pituitary of the female rat at various stages of the reproductive cycle was studied by Andersen, Prest & Victor. The animals were killed at the eighteenth day of pregnancy, at parturition or after they had been lactating 27 days. Comparison was made with rats taken in pro-estrus, estrus, di-estrus and after ovariectomy. The mean respiration rate and R.Q. of liver were higher in pregnancy than in any other condition except lactation (R.Q.). Significant differences were found between the metabolic rates of anterior pituitaries (taken whole) of pregnant, parturient and lactating rats. These changes were associated with differences in gland weight. There were no significant differences in the aerobic and anaerobic glycolytic rates. An explanation of the increased rate of metabolism in the anterior pituitary during the estrus cycle and at parturition is afforded by the observations of Victor & Andersen (1) showing that *in vitro* theelin and dihydrotheelin and *in vivo* (in spayed rats) dihydrotheelin, had the effect of in-

creasing the oxygen consumption of the anterior pituitary. Andersen & Victor found the respiratory metabolism of the liver of spayed rats significantly increased three days after treatment with injections of amniotin or theelin, but on the fourth day it returned to the original level. The metabolism of kidney was unaltered. The effect on liver, unlike that on anterior pituitary, occurred only *in vivo*. The effect on liver also was abolished by hypophysectomy as well as by thyroidectomy [Victor & Andersen (2)]. Victor & Andersen (3) later found that removal of the ovaries caused a depression of oxygen absorption in rat-liver tissue of 19 per cent, but was without effect upon that of the kidney cortex. Removal of the ovaries plus removal of the thyroid caused a depression of 25 per cent in the rate of oxygen consumption by the liver, and was without effect upon that of the kidney cortex; removal of the hypophysis caused a depression in the oxygen consumption of liver of 30 per cent, and in kidney of 20 per cent. Changes in liver metabolism paralleled those in basal metabolism under the conditions named. The R.Q.'s of both liver and kidney were lowered by spaying and thyroidectomy. Hypophysectomy lowered both still more. Infection caused an increase in metabolism as well as in R.Q. of the liver in all experiments.

Brouha-Dubuisson studied the respiratory metabolism of ovarian tissue of the rat and found distinct differences according to whether one compared the prepubertal with the adult ovary, or different steps of the sexual cycle. The  $Q_{O_2}$  was low in the infantile animal but rose little by little until puberty when a notable increase began. Respiration was lowest in pro-estrus, and increased progressively through estrus and metestrus to di-estrus when it reached its highest value.

*Other endocrine factors.*—The effect of injected cortin upon oxygen consumption in twelve normal human subjects is reported by Hitchcock, Grubbs & Hartman. Oxygen consumption while standing was reduced on the average 6 per cent and while walking on a treadmill was reduced in ten of twelve subjects an average of 10 per cent, "part of which might have been due to training." The effect was not always parallel to hormone content. Repeated injections seemed to produce reversal of the effect on two subjects. An extension of these experiments is found in the work of Missiuro, Dill & Edwards who employed four human subjects. Injection of 40 to 80 cat units daily for five days produced no constant or

striking effect on the B.M.R. The efficiency with which easy walking on a treadmill was accomplished was increased for some days after injection. The capacity for anaërobic work was not increased. A notable effect in moderate or severe work was a decided lowering of blood pressure in the early stages of recovery. Cortin, according to the observations of Kaunitz & Selzer, prolonged the "oxygen-debt" period observed in the diaphragm of rats previously fatigued excessively. The livers of these rats, fatigued and treated with cortical hormone in the majority of cases, did not exhibit any "oxygen debt" as they do in fatigued normal animals.

Adrenalectomy has long been known to reduce the B.M.R. Carr & Beck from a large number of determinations on ten rats, before and after the double operation, found that the average reduction in total metabolism was 25 per cent. Schaeffer & Pollack have shown that a correlation exists between the rise of energy metabolism and the fall of plasma lipids produced by injections of epinephrine into rabbits.

Kochakian by the use of testosterone and testosterone acetate, has confirmed his previous observation that androgens do not increase the energy metabolism of castrated dogs, although the doses given were sufficient to cause marked nitrogen retention. Slight increases in the basal metabolism of eunuchoid men treated with testosterone propionate are reported by Kenyon, Sandiford *et al.*

Siegmund & Flohr, investigating the influence of insulin upon the combustion of alcohol in human subjects, found that with medium doses of the intoxicant, the hormone in doses of about 40 units increased in general the metabolism of alcohol. The affinity of insulin for alcohol, however, was not so great as for sugar. There was no mitigation of the intoxicating effect of alcohol. Medium doses of insulin with large doses of alcohol are not suitable as a sobering agent. Sheldon, Johnston & Newburgh have confirmed once more the observation that diabetics with known limited tolerance cannot, unaided by insulin, oxidize glucose in excess of this limit nor did the previous use of insulin by one subject maintain his higher oxidative ability twelve hours after it was discontinued.

Mizuno & Hirota found that extirpation of the thymus from male guinea pigs caused a decrease in oxygen consumption and an

increase of R.Q. Both gradually returned to normal. Feeding thymus tissue produced the opposite effect in each instance. Submaxillary gland tissue of the cat produced at rest an R.Q. of 0.59 to 0.80, according to Deutsch & Raper. Its metabolism *in vitro* was increased considerably by acetylcholine with a minimal amount of eserine. The characteristics of this metabolism are different from those of the resting tissue.

*Hypermetabolism<sup>5</sup> of fever.*—Numerous observations on the "basal metabolism" of fever have shown that almost without exception the elevation of body temperature sets in with a more or less marked increase of oxidation. Within recent years, however, it has been shown (cf. Grafe) that infections, independently of fever, can produce the same changes in metabolism. The explanation of this effect is not clear. Schäfer-Fingerle used "Pyrifer," "Sufrogel," "Artigon" and autovaccine injected parenterally to produce artificial fevers in man. He found in twelve out of thirty-six cases that the increase in oxygen consumption preceded the onset of "fever" (rectal); in fifteen the increase of temperature and metabolism occurred simultaneously; in seven there was no increase of either and in two the increase came so late that its course could not be followed. The general effects were the same whether the increased metabolism occurred independently of the fever or at the same time. The experiments throw no light on the significance of the accelerated metabolism.

Awareness of the same distinction between temperature and metabolic effects from infection is evident also in the work of Johnston & Maroney who determined the B.M.R. before and after tonsilectomy. Removal of infected tonsils produced a fall in metabolism and, coincidentally, improvement in nitrogen balance. The latter was out of proportion to any change in protein metabolism that might be accounted for as a result of the change in basal metabolism.

The effect of pulmonary tuberculosis on metabolism depends on the stage of the disease and the secondary effects, according to Ikari. At the beginning of infection the B.M.R. is most often

<sup>5</sup> The allocation of the metabolism of fever to basal rather than to super-metabolism gives recognition to the fact that other factors than temperature are at work. Also it makes a concession to practise, for all clinicians speak of "doing basals" on their febrile cases.

increased and in its late form decreased. In patients with pleuritis the B.M.R. is considerably increased at the beginning and returns to normal with healing of the disease. In all of these diseases, in Ikari's view, the augmentation of the B.M.R. is caused, in large degree, by hyperfunction of the vegetative nervous system.

*Other causes of hypermetabolism.*—Continuing their studies of the effects on energy metabolism of low-salt diets, Kriss & Smith (1) observed that depleted rats, in periods unaffected by activity, almost invariably produced more heat than their litter-mate controls on adequate salts. Rats on the deficient diet weighed less and the increase in heat production due to spontaneous activity was less than for the controls. During the second and third months of the experiment the R.Q.'s of the mineral deficient animals were significantly lower than those of the controls [Kriss & Smith (2)]. There was no effect on protein metabolism. The increase of energy metabolism was entirely basal, the specific dynamic action not being affected. Fat-starved rats, because they remain smaller than their normal controls, have a higher basal metabolism per unit of surface area (Burr & Beber).

Carleton & Fenn have shown that the excess respiration of frog muscle, caused by replacement of nitrogen of the air by carbon monoxide (Fenn & Cobb, 1932), is little if any modified or decreased by the hypermetabolism produced by potassium chloride. Bodine & Boell believe that the stimulated respiration of the grasshopper embryo, caused by 2, 4-dinitrophenol and 3,5-dinitro-*o*-cresol, is mediated through the normal respiratory mechanism which is injured by cyanide and carbon monoxide.

#### SUPERMETABOLISM<sup>6</sup>

*Specific dynamic action (S.D.A.) of protein.*—One of the most significant contributions in several years is the review of Schaeffer & Le Breton. Part I sets forth the original conception of Rubner, the modes of expression of S.D.A., its calculation, the different modes of administration of amino acids and critiques of the theories of Rubner as extended by Terroine & Bonnet, of Grafe, Aubel, Geelmuyden, Borsook and the opinion of Lusk. A chapter is

<sup>6</sup> Due to the exigencies of space it has been necessary to restrict the discussion of supermetabolism to the phenomena of the specific dynamic action of protein, and the influence of temperature.

devoted to the question of kidney work, as originally conceived by Zuntz and as elaborated in recent years by Borsook and associates. The determinative work of Dock, of Rein, of Dombrowski and of Van Slyke and associates prove the independence of oxygen consumption and the secretion of urea. (See also p. 154.) The last chapter contains a brief discussion of oxidation-reduction reactions. Part II contains many experiments hitherto unpublished by the authors and their associates as well as the salient findings in several contributions from the Strasbourg laboratory which have appeared elsewhere. Thus Dontcheff & Schaeffer (1) found that a rabbit given a certain dose of peptone by stomach tube on two successive days, usually exhibited a rise of oxygen absorption which was of lesser magnitude and of shorter duration on the second day. Rabbits also were subjected to low environmental temperature for a few hours after the peptone meal and then returned to the state of thermal neutrality until the S.D.A. disappeared [Dontcheff & Schaeffer (2)]. The latter part of the heat curves coincided fairly well with those obtained on the same animals a day earlier at thermal neutrality throughout the experiment. Calculations showed that if the animal had produced heat in response to cold at the expense only of the S.D.A. of the peptone this would have been exhausted by the several hours in the cold environment and no S.D.A. should have remained thereafter. Since it did remain in full force, the authors conclude that the S.D.A. of protein is not interrupted in its course by the action of thermogenetic hormones (epinephrine and sympathin).

The authors have frankly embraced the theory which ascribes S.D.A. to stimulation of the autonomic nervous system. Schaeffer, Dontcheff & Le Breton have repeated on rabbits experiments which Lips did in Noyons' laboratory on cats, *viz.*, to show that some months after double splanchnicotomy the S.D.A. of protein falls to a very low point as compared with the effect in normal animals [Lips obtained complete disappearance in cats (cf. Noyons)]. They found also, as did Lips, that ergotamine tartrate ("gynergène") in doses which paralyzed the sympathetic system diminished the S.D.A. of proteins for the duration of its paralyzing action. They believe that excitation by the amino acid split products liberates into the blood one or more hormones (epinephrine, sympathin) which are responsible for the increased heat production following ingestion of protein. Space does not permit a fuller

presentation of the argument. It strikes the present writer that evidence should be adduced that double splanchnicotomy and ergotamine tartrate do not interfere with absorption of protein from the gut. If it were merely retarded strongly S.D.A. might be greatly reduced.

Urethane, according to Le Breton & Schaeffer does not inhibit the oxidative changes underlying the S.D.A. of protein and therefore the latter belong to either type I or type III of Dixon. The oxidation of alcohol on the other hand, according to Dawborn & Le Breton, is of the dehydrogenase type and is regularly inhibited by urethane.

Additional support for the neurogenic theory of S.D.A. comes from Rothschild. The drug piperidine-methylbenzo-dioxane, prepared by Fournau and designated as "933F," is known to block the orthosympathetic system, thus preventing a central stimulus from reaching the adrenal gland. In company with Wolfermann, Rothschild used this agent on a dog on whom the S.D.A. had been measured following a meal of 110 to 140 gm. of lean beef. Given alone, intraperitoneally, the drug produced an increased metabolism even greater than the S.D.A. of the meat, but when given at the same time as the meal the two effects did not summate. In fact the supermetabolism was only a little greater than that of the S.D.A. It was therefore impossible to draw any conclusion as to the rôle of the autonomic nervous system. Rothschild himself, however, next tried the drug on cats anaesthetized with dial which is known not to prevent the S.D.A. In control experiments "933F" did not raise the metabolism materially above the narcotic level. Casein was then given simultaneously with the drug to animals already narcotized and no S.D.A. appeared. No evidence is given that the protein caused hyperaminoacidemia. Nevertheless, "the facts lead to conclusions regarding the decisive rôle which the autonomic nervous system must play in the mechanism of this so-much controverted phenomenon" of the S.D.A. of protein.

Löw & Kréma have given attention to this important matter of amino acid concentration in the blood in relation to S.D.A. following meals of protein (ham) in metabolically healthy subjects, in obesity and in pregnancy. In metabolically healthy persons, following injection of anterior pituitary extract ("antephysan" Richter), they also determined the increased metabolism and the hyperaminoacidemia. In all cases when there was no increase in the



amino acid content of the blood there was no S.D.A. The increased metabolism ran parallel in a general way with the hyperaminoacidemia. The conception is developed that the S.D.A. results from stimulation of the hypophysis-diencephalon system by the exogenous amino acids in the blood and there results an endogenous hyperacidemia eventually responsible for the increased metabolism.

Directly opposed to these results of Löw & Krčma are those of Szakáll obtained on dogs. Glycine, glutamic acid and sodium acetate were injected intravenously. There was no parallelism whatsoever between the supermetabolism and the extent of nitrogen excretion or the deamination as measured by the blood or urine urea nitrogen. The supermetabolism began earlier and was already falling when deamination was at the high point. About 50 per cent of the amino acids was excreted unchanged. The author explains the S.D.A. as the result of irritation of the liver, the mechanism of which he discusses.

Atzler, Lehmann & Szakáll (2) studied the effect of caffeine on carbohydrate and protein metabolism in the tracheotomized dog, connected with a Benedict respiration apparatus. It was found that the effect of sugar in sparing phosphates [Atzler, Lehmann & Szakáll (1)] was to a large extent nullified by caffeine. Caffeine, therefore, inhibits the restitution of the phosphorylated compounds in the recovery processes after work. The respiratory quotient fell slowly after carbohydrate and caffeine indicating interference with the normal utilization of carbohydrate in the recovery processes. Caffeine also depressed the capacity of the liver to deaminate amino acids. Both after ingestion of meat and after intravenous ingestion of amino acids the retention of amino nitrogen was increased. Notwithstanding these changes caffeine increased the S.D.A. of protein. It would seem therefore from these results that S.D.A. is not associated with the processes of deamination.

Following the procedure of Kriss, Forbes & Miller, which takes the energy metabolism of nitrogen and energy equilibrium instead of fasting as the base line, Kriss made definite and uniform additions of casein, gelatin and dried heart muscle to the maintenance diet and called the difference in calories produced the S.D.A. In computation of the thermogenic effect of the supplements he made use of the new factors for calculation of the heat production of

protein derived by Kriss & Voris for the same proteins. Casein showed the greatest and heart muscle the smallest dynamic effect, expressed either as total calories or as percentage of the metabolizable energy. No significant differences were observed between the specific dynamic values of the several proteins fed in different amounts when expressed as percentage of the protein calories fed. This agrees with the older results of Rubner and Lusk. Barott, Fritz, Pringle & Titus also found the S.D.A. of casein in chicks higher than that of gelatin. It was more than twice as high in very young chicks as in chickens ten to twenty weeks old, which seems remarkable.

Forbes, Voris, Bratzler & Waino have carried further the study begun by Forbes, Swift, Black & Kahlenberg on the utilization of energy-producing nutriment and protein as affected by the plane of protein intake. The earlier study covered the range 10, 15, 20 and 25 per cent (by weight) of protein in equicaloric diets, while the present one extends the range to 25, 30, 35 and 45 per cent. In a discussion of the results of both studies over the range from 10 to 45 per cent protein the authors concluded that as food protein increased digestibility increased but metabolizability of food energy decreased, heat production decreased, and wastage of food energy in the urine (but not in the feces) increased. Up to the optimum point of 25 per cent protein intake body weight and fat increased, but with further increase of protein the gains in body weight, body energy, nitrogen retention and fat per gram of nitrogen retained decreased slightly. As the heat production, both in absolute units and as percentage of food energy, declined at a uniform rate from the level of 10 per cent to 35 per cent protein and increased only a fraction of a per cent from 35 to 45 per cent, the authors conclude that the separate specific dynamic effects of the foodstuffs are damped out in mixed diets during the growth period.

In the sense that it provides a method of determining the heat production in relation to food energy the plan of experimentation recently described by Forbes, Swift & Black also should be included under the heading of S.D.A. The method is calculated to furnish much additional information outside as well as inside the field of energy metabolism. While it harks back to the old method of carbon and nitrogen balance introduced by C. Voit about 1866, the authors have amplified and improved upon that method to

make it essentially new. New equipment which, in multiple, provides for simultaneous measurement of carbon and nitrogen intake and output in six small animals like rats, is described. One procedure consists of growth experiments in the course of which a series of carbon and nitrogen balances can be struck at intervals upon the same food intake. It yields results in terms of gross efficiency of diets, covering requirements for maintenance and production of body gains. A second procedure consists of nutritive measurements also by means of carbon and nitrogen balances, representing the net energy point of view. Feeding at maintenance and a higher level are compared with fasting, and heat increment (S.D.A.) as well as net energy can be obtained for the two levels of feeding.

Much has been written, pro and con, regarding the dynamic action of the ternary intermediate compounds. Oberdisse & Paraskevopoulos have come forward with evidence that the non-nitrogenous intermediary substances which are supposed to be formed in the metabolism of alanine, namely, pyruvic acid, acetaldehyde and acetic acid, as well as alanine itself, exert a dynamic effect when perfused through the surviving liver of the dog. Lactic acid and glycolic acid had no such effect. Propionic acid as well as acetic acid raised the oxygen consumption. Oberdisse & Eckardt previously had shown that the kidney in a heart-lung-kidney perfusion system likewise responded to amino acids with an increased absorption of oxygen, which was not related to urine formation. In the present report there was no parallelism between dynamic effect and urea formation from alanine. The observations of Eaton and associates that heat production in man is not increased by ingestion of twenty to thirty grams of urea, although trebling the work of the kidney, have been confirmed by Rajzmann and by Carpenter. The former injected urea solutions parenterally into rabbits and rats and the latter fed thirty to forty grams of urea to human subjects without in either case raising the oxygen absorption. Eaton's still more convincing demonstration by nephrectomy experiments that the kidney does not play any large part in the S.D.A. of glycine has pretty well disposed of Borsook's theory (see p. 150). Unilateral nephrectomy results in increased blood flow through the remaining kidney, which reaches the volume of the combined flow through both kidneys within 3 months

(Levy & Blalock). Renal oxygen consumption runs proportional to blood flow. After constriction of the renal arteries to produce experimental hypertension Levy, Light & Blalock observed the same proportionality.

Cera & Lombroso, stimulated by the conclusions of Wilhelmj & Mann and of Stassi concerning the effects of fasting on the S.D.-A. of amino acids, tried the effects of whole protein on normal and fasted dogs. Feeding meat to a dog fasted for six or seven days resulted in a specific dynamic effect which was less in every instance than the effect produced by the same meal on the same dog before fasting. The authors raise the question whether this result, in contrast with those of Wilhelmj & Mann, may not be explained by a lesser degree of proteolysis after fasting. If it were to stop at the polypeptide, rather than the amino acid stage (a question which implies, perhaps, slow recovery of secretion of proteolytic enzymes), the facts would be explained.

A question of considerable current interest is the fate of perfused blood protein. Martini asserts that perfusion of blood into the veins of surviving animals ordinarily does not increase the metabolism; but sometimes in animals maintained on a protein-rich diet, the oxygen absorption is increased by amounts corresponding with the S.D.A. of an equivalent meal of protein given orally. Does this mean that circulating proteolytic enzymes break down the surplus protein?

What would happen if an aviator flying at high altitude ate a heavy protein meal as a protection against cold? Giaja (2) believes he has shown in previous publications (1) that certain of the oxidations of the body (basal, the effect of dinitrophenol and of  $\beta$ -tetrahydronaphthylamine) are independent of oxygen tension, while others (those set in action by hibernation, fever, and chemical regulation to cold) are dependent. He now applies the same test (chamber with low atmospheric pressure maintained at thermal neutrality, 32 to 33°) to the supermetabolism of S.D.A. in rats. In a report of only three fasting experiments and three after food he concludes that lowering the atmospheric pressure to 380 mm. does not affect the oxygen absorption of fasting, but after a meal of peptone it completely obliterates the dynamic effect of the food. Laser did not find the uptake of oxygen by tissue slices reduced by oxygen tensions of 5 to 20 per cent as compared with

100 per cent in the manometric system. R.Q.'s however were lower and aerobic glycolysis was higher at low tensions, changes which are ascribed to altered enzyme activity.

A diurnal rhythm in the energy metabolism of the albino rat discovered by Herring & Brody may explain some failures to observe the S.D.A. of foods. The total metabolism showed an ascending rate beginning about noon, reached a maximum about 9 P.M. and declined thence to noon again. Continuous illumination with divided feedings (one-eighth of a day's food every three hours) extinguished the rhythm in about a week. Burr & Beber found a marked diurnal cycle of activity of rats which seemed to be independent of light. They believe that their experiments on fat-deficient rats receiving carbohydrate indicate that the S.D.A. was associated with activity of the digestive organs (old *Darmarbeit* of Zuntz) rather than with intermediary changes of metabolism. An interesting diurnal curve in the rate of oxygen consumption of fasting young chicks is described by Barott, Fritz, Pringle & Titus. Its amplitude was greatest in very young birds and decreased rapidly with age.

#### SUPERMETABOLISM CAUSED BY TEMPERATURE

For quite a time when diathermy became the rage it was thought that high frequency currents must have some specific effect on metabolism aside from the fever produced. Nasset was one of the first to discredit this idea. A new study by Bergh & Krarup on human subjects exposed to bombardment by radio waves gives the same answer. The authors entertained no illusions regarding the measurement of actual energy input, but choosing arbitrary empirical values gave doses at a fixed wave length of 14 m. The increased metabolism found was of the same order of magnitude as that found in hyperpyrexia produced by other means. The average increase in heat production computed per 1° C. rise of rectal temperature was 10.1 per cent.

*Adaptation to cold.*—Schwabe, Emery & Griffith report some interesting observations on this phenomenon obtained with their new apparatus in studies on rats (Schwabe & Griffith). The animals were exposed to an environmental temperature of 7° to 12° C. for two-thirds of the day and then given relief at 29° C. for the remaining third, at which temperature also the respiratory metabolism was determined. This procedure gradually raised the basal

metabolism 11 to 16 per cent, the maximum being reached between fifteen and thirty days. The R.Q. was not affected by the hardening treatment. The excellent work of Hardy & DuBois on human subjects is concerned mainly with the partition of heat loss and as such belongs under "Heat Regulation" (cf. p. 109). It is worthy of note in this review, however, that they found no change in heat production, as calculated from the respiratory exchange and urinary nitrogen, over a range of environmental temperature from 22° to 35° C. notwithstanding that the subjects were nude or nearly so, and considerable tensing of the muscles was felt at the lower temperatures. Hardy, Milhorat & DuBois also found no increased heat production when nude subjects were exposed to the draft of an electric fan. Winslow, Herrington & Gagge (1), curiously enough, found the metabolic rate essentially constant over a range of 18° to 41° when subjects were nude, but observed (2) a chemical regulation when subjects were clothed.

*Hibernation.*—An animal accustomed to adapt its heat production to the environmental temperature certainly would not behave as did these nude men. Even animals which hibernate can perform homeothermic adaptation in summertime as shown for the spermophile (European gopher) by Gelineo (1). Giaja (1), however, has shown that this process of chemical regulation (Rubner) is disturbed by low oxygen tension, although the basal metabolism is not. Chevillard, Hamon & Mayer found that the level of oxygen tension just sufficient to maintain homeothermy in the mouse depended on the external temperature; thus at 30° C., 8.5 per cent of oxygen in the atmosphere was sufficient, while at 20° C. 12.8 per cent, and at 4° C., 20.5 per cent, respectively, were necessary.

Gelineo (2) followed the heat production of the spermophile from late September to late November, and found a progressive diminution of thermogenesis in response to cold, although the environmental temperature was practically constant. Some days before torpor set in the B.M.R. declined also. It is this diminution in the power of thermogenesis to cold which eventually brings on the winter sleep. Benedict & Lee, in the most significant study of hibernation which has appeared in many years, have confirmed this conception by their numerous observations on the American marmot or woodchuck. Previous to resigning himself to hibernation the marmot may pass through several cycles of perfect chem-

ical regulation. But as the outside temperature falls still more this mechanism proves inadequate and deep stupor, below the frost line in the ground, economizes on food and heat production. Still lower temperatures however may cause the animal to waken. In agreement with Kayser (3) for the spermophile the authors obtained values for the fully awakened marmot approximately 100 times that of deep hibernation.

The R.Q.'s in this extensive study of the hibernating marmot were found also to agree with those obtained by Kayser (3) for the spermophile and European marmot and for the hamster (Kayser & Dell), namely, in the neighborhood of 0.70. The guinea pig [Kayser(1)] subjected to only 10° C. produced heat from fat unless he shivered when the R.Q. went up to 0.8 or higher. The same observation held for the rat and pigeon [(Kayser (2))]. Benedict & Lee point out that in deep winter sleep a difference of only 0.1 cc. of oxygen per minute for a marmot of 2 kg. weight makes the difference between an R.Q. of 0.64 and 0.70. If this continued for four hours and all the oxygen went to the formation of glycogen from an oxygen-free source, the oxidation of the glycogen would furnish only 7 per cent of the energy produced in a 20-minute waking-up period. With a single animal the authors found in 25 measurements by the most refined methods possible that the R.Q.'s in deep sleep varied between 0.68 and 0.79, with the average at 0.71. During a waking-up period while the heat production was advancing from 28 kilocal. to 2230 kilocal. per sq. m. ( $10 W^{2/3}$ ) the R.Q.'s rose in successive periods as follows: 0.70, 0.77, 0.79, 0.78, 0.80, 0.80, 0.79, 0.79, 0.77, all of which could be accounted for by combustion of protein and/or liberation of carbon dioxide as the tissues became warmer.

The relation of the thyroid to thermogenesis to cold was investigated in the goose by Lee & Lee. Deprived of the thyroid this animal produced the necessary amount of heat to maintain its body temperature very near that of the normal animal under like conditions. The basal heat production at thermal neutrality, however, was 33 per cent below normal.

The adrenal gland is essential to chemical regulation. Horvath, Hitchcock & Hartman found that thermogenesis in the rat at 4° C. was very much retarded when one adrenal had been removed. Still greater deficiency was observed by Horvath in doubly adrenalectomized rats. Whereas normal rats at 4° C. exhibited a super-



metabolism of 176 per cent over that at 29° C., the loss of both adrenals reduced the response to +79 per cent. Post-operative colonic temperature in the cold was maintained for only about four hours. Ring, in his experiments, left only a small part of one adrenal cortex. Body temperature was maintained in the cold although the response was somewhat sluggish. The  $\mu$  value of Arrhenius for oxygen absorption was 16,100 and appeared to be higher for falling than for rising body temperature.

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DEPARTMENT OF VITAL ECONOMICS  
UNIVERSITY OF ROCHESTER  
ROCHESTER, NEW YORK

## THE PERIPHERAL CIRCULATION\*

BY H. C. BAZETT

*Department of Physiology  
University of Pennsylvania, Philadelphia, Pennsylvania*

It is impracticable to cover comprehensively the immense literature of this diffuse subject. This review is limited to selected papers published in 1937 and the first half of 1938 with occasional references to earlier work. Some articles, which are not here discussed, are cited in the bibliography; these are there distinguished by the insertion of their titles.

### PHYSICAL FACTORS IN THE CIRCULATION

A considerable number of papers have been concerned with the determination of the elastic properties of vessels either by measurement of the pulse wave velocity in life or by direct measurement of elasticity after death. The rate of wave transmission is dependent on the volume-elasticity constants of the system. In excised vessels often the measurement made has been by the elongation of a transverse strip of vessel wall, when subjected to stress (15), rather than the determination of the volume elasticity. The latter cannot be predicted accurately from the former, since longitudinal stretch is not negligible. Consequently recent direct measurements of the more important volume-elasticity coefficients are valuable. From such measurements the elastic relationships of stress to strain in the transverse fibers of the wall may be approximately calculated.

The volume-elasticity coefficient is complicated by the geometrical properties of cylindrical tubes. Wiggers (16) has performed a service in demonstrating the fact that, owing to these properties, the increase in pressure in an aorta resulting from any given stroke volume is less, rather than greater, the higher the pressure levels, provided that abnormally high pressures of 200 mm. Hg or more are not attained. This he demonstrated directly by connecting an artificial pump with the thoracic aorta of a recently killed dog. The explanation lay in the S-shaped volume elasticity curve given by the aorta. At intermediate pressures it was more distensible than at lower pressures. The decreased pulse pressure was attrib-

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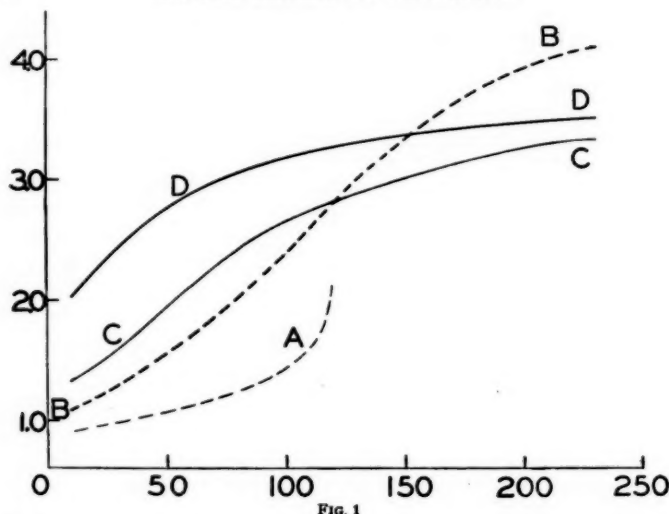


FIG. 1

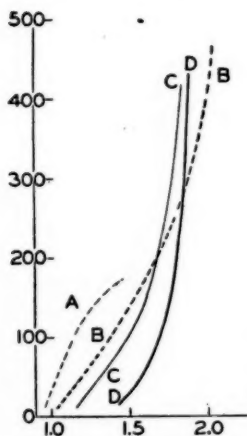


FIG. 2

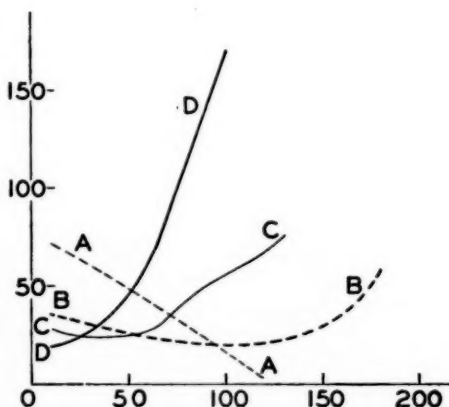


FIG. 3

## DESCRIPTION OF FIGURES

FIG. 1.—Volume elasticity curves of condom rubber (A), and of aortas of individuals of 20 to 24 (B), of 36 to 42 (C), and of 71 to 78 years old (D). The latter three curves are taken from the paper of Hallock & Benson (17). Abcissae: pressure in mm. Hg for curves B, C, and D, and in mm. H<sub>2</sub>O for curve A; ordinates: volume per cm. length for curves B, C, and D, and per mm. length for A. (The scales for A retain these differences throughout Figures 2 and 3.)

FIG. 2.—The approximate relationships of the transverse tension of the wall to increase in tube circumference for the curves of Fig. 1. The square roots of the volumes of Figure 1 are plotted as abscissae and these square roots multiplied by the pressures as ordinates.

FIG. 3.—Curve A, the pressure change predictable from Figure 1 for a given increase in volume of a condom at varying diastolic pressures (increase of 38.5 per cent of the volume at 10 mm. pressure); diastolic pressures are plotted as abscissae and predicted pulse pressures as ordinates. Curve B is a curve similarly derived for the aorta of a young subject. Curves C and D indicate the pulse pressure predicted for the same absolute increase in volume as that of curve B (0.41 cc. per cm. length) in middle-aged subjects of 36 to 42 years (Curve C) and in senile subjects of 71 to 78 years (Curve D).

uted to "the progressive increase in capacity and reduction of the ratio, systolic discharge: aortic capacity." The latter is not the whole explanation, however, for the volume-elasticity curves demonstrate that the pulse pressure must become smaller even if this ratio is unchanged.

The data described by Wiggers are predictable from the volume-elasticity curves of human thoracic aortas described by Hallock & Benson (17). In spite of the longer time after death these aortas show elasticity curves comparable to those obtained in the recently killed dog. Three of these curves (17) are reproduced in Figure 1 as well as, for contrast, the volume-elasticity curve for a highly distensible rubber tube (condom). Curves A (condom) and D (senile aorta) are quite different; curves B (young adult aorta) and C (middle aged aorta) are S-shaped and intermediate in type. If longitudinal stretch is neglected, the relation of transverse tension to length of the circumference may be shown in arbitrary units by plotting the square root of the volumes multiplied by the pressures as ordinates against the square roots of the volumes as abscissae (the transverse tension varies with the pressure multiplied by the radius; both the latter and the circumference vary as the square root of the volume). The values so derived from Figure 1 are plotted in Figure 2. The significance of the lines depends on their slope. The real transverse stress to strain relationship is more truly represented by plotting the ratio  $d\sqrt{v}/\sqrt{v}$  against  $P\sqrt{v}$ . For the condom this is practically a straight line. The senile aorta shows a rapidly increasing transverse elasticity coefficient dependent on its loss of elastic tissue and the influence of the resistance of white fibrous tissue (17). The younger aortas show transverse elasticity coefficients that slowly increase as the effects of the varying components of their walls overcome those due to the properties of tubes. The derived curves of Figure 2 agree with the familiar ones which relate the tension of an isolated strip of vessel wall to its length.

From the curves of Figure 1 the pulse pressure likely to result from any given increase in volume may also be deduced. The pressure changes so estimated at varying diastolic pressures are shown in Figure 3.<sup>1</sup> Curve B for the young aorta is in thorough agreement

<sup>1</sup> In the curve representing distensible rubber tubing only the lower values of distension are indicated. At greater distensions the pressure change for an increase in volume is negative.



with the experimental data obtained on the dog's aorta (16). The great reduction of pulse pressure at higher pressures for any given stroke volume in a simple distensible tube, such as a condom, is dependent on the properties of a cylinder: (1) the greater the initial radius the less the increase in circumference necessary to accommodate any given volume (expressed mathematically in a cylinder of volume  $V$ , radius  $r$ , and length  $l$ ,  $dr/r/dV = 1/2\pi lr^2$ ); (2) the greater the radius the more effective is any pressure change in stretching the wall (expressed mathematically this factor depends on the relation of transverse tension per unit cross-sectional area of the wall ( $\sigma_t$ ) to the radius ( $r$ ) and thickness ( $a$ ) of the wall, and to the pressure ( $p$ ) according to the equation  $\sigma_t = pr/a$ ; changes in the thickness of the wall have been here neglected). In the aorta these factors are counteracted to an extent varying with age by the increase in the modulus of elasticity with stretch. A decreased pulse pressure with constant stroke at increasing degrees of distension was demonstrated by Wiggers (16) also in rubber tubing distended by a pump. In these schema experiments in this pressure range the relation of volume increase to pressure increase was described as linear. Owing to the properties of tubes already described, any strict linearity of the sort is improbable. The accuracy of these volume elasticity curves must be questioned.

These two papers and the curves derivable from them are emphasized because of their physiological and pathological importance. The enlargement of the aorta with age, though known for many years, has recently received attention (17, 20, 21, 22, 23), for as may be seen in Figure 3, it compensates for loss of arterial elasticity, provided that diastolic pressure is low. A normal stroke volume may then even be accommodated with less than the normal pressure change. The complexity of the relations should be a warning against over-confidence in estimations of stroke volumes from pulse pressures (16); on the other hand it is remarkable how flat is curve B of Figure 3 within the common blood pressure ranges. Hallock & Benson (17) show that the pulse wave velocities calculable from the volume elasticity coefficients of sections of aorta are in close agreement with those observed in life.

Numerous papers attempting to analyze circulatory conditions by pulse wave velocity measurements have appeared from the Munich School. Following injection of epinephrine and sympatol the pulse wave velocity has been found not to be predictable from

the blood pressure level. Even in the aorta the condition of the muscular coat exerts an important influence [Wezler & Böger (18)]. At similar pressure levels the pulse wave velocity is slower following sympatol than after epinephrine injection. This is interpreted as due to an increased distensibility (lowered modulus) from contraction of the muscular coat. The conclusion is drawn not only that contraction of the muscular coat increases the distensibility of the vessel, but also that a decreased distensibility of the large vessels in essential hypertension is due to decreased contraction of the muscular coat (19). From studies of pulse wave velocity following meals and exposure to temperature changes Bazett and his coworkers conclude also that even in the aorta the contraction of the muscular layers is important, but reach the opposite deduction that such contraction is associated with decreased distensibility (20 and 21). Neither view is established by direct evidence. In view of the effect of mere reduction in diameter on the distensibility of tubes, the latter interpretation is the more probable.<sup>2</sup>

Wezler & Böger have modified Broemser & Ranke's equation for calculating stroke volume from pulse pressure and pulse wave velocity (22, 23). The apparent natural frequency of the vascular system as determined by the time interval between the peak of the primary wave of the femoral pulse and the peak of the second wave in this pulse is utilized, as well as the pulse pressure, the pulse wave velocity from the subclavian to the femoral and the predicted size of the aorta. The time measurement on the femoral pulse cannot be made accurately, and is often confused by the fusion of a dicrotic wave of central origin with the reflected wave. There are no adequate grounds for assuming that the effective length of the elastic reservoir is one-quarter of the wave length (*i.e.*, that the system behaves like a tube open at one end and closed at the other and shows reflection with change of sign). The determination of a single pulse wave velocity does not allow an estimate of the elas-

<sup>2</sup> Direct evidence has now been advanced by C. J. Wiggers & R. Wégria [*Am. J. Physiol.*, **124**, 603 (1938)] that contraction of the aorta can occur and that it is associated at pressures above 150 mm. with an increased distensibility of the aorta. Their curves also demonstrate that at pressures below 125 mm. the decrease in the diameter results in a larger pulse pressure for a given absolute increase in volume. The conclusions, therefore, both of Wezler and Bazett are confirmed at the pressure levels with which they deal.

ticity of the whole system. An agreement of the average estimates for cardiac output so obtained with the average estimates by gas methods is no proof of accuracy. It is claimed (23) that the blood stored in the aortic system during systole and released in diastole is equal to that leaving the arteries during systole, and the work of Bazett *et al.* in 1935 is quoted as confirming this statement. Such an equality cannot be assumed. As far as can be judged (unpublished details of experiments reported in papers 20 and 21) the former may be double the latter at fast, and less than three-quarters of it at slow, pulse rates. Some credence must be given to these estimates for the total stroke volumes so calculated were confirmed by simultaneous measurements of the stroke volume by acetylene. Wezler & Böger's individual estimates and calculations cannot be accepted as proven.

On the foundation of these papers the Munich School have built up a large literature analyzing the resistance (24) and resting condition (25) of the vascular system, the effect of sympathetic stimulation (26) and the like (27). If the premises be not accepted, the deductions remain unproven, though the data may be valuable. There is no doubt that if the cardiac output and mean blood pressure are known, even approximately, a good estimate may be made of the effective peripheral resistance of the whole system (20, 21, 24). Such resistance may be expressed, if desired, in absolute units (dynes  $\times$  seconds)/cm<sup>5</sup> (24), but such expressions give a false sense of security. Owing to the complexity of the system and the varying viscosity of blood in different sized tubes the resistance cannot be analyzed adequately and some arbitrary unit is more commensurate with present knowledge.

The pulse wave velocity in the leg arteries has been measured at varying effective internal pressures by compressing the leg with a Riva Rocci cuff (28, 29) according to the method of Bramwell & McSwiney (29a). From these measurements the distensibility of the vessel has been estimated. This is found to vary considerably in young individuals, but not to be significantly different either in old or hypertensive subjects. Such results differ from those obtained by Böger & Wezler (19). The latter authors considered the peripheral pulse wave velocities higher than those observed in normal subjects with blood pressures raised to a similar extent by epinephrine. On the other hand Steele (30) concludes that the pulse wave velocities do not exceed those predictable from the pressure

level. The peripheral pulse wave velocity in young hypertensives is very variable even in the same subject (31).

Hauck (32) has made simultaneous measurements by oscillographic methods of the pressures in the brachial and finger vessels. He finds these pressures not significantly different (less than 10 per cent) in contrast to the earlier conclusions of von Bonsdorff (32a). He does not state the thermal conditions of the experiments. This factor may play an important role. While he confirms the work of previous workers that the peripheral pulse wave from brachial to radial is faster than in more central vessels, he finds this velocity reduced again between the radial and finger to 5.2 to 5.8 meters a second. There is therefore a highly elastic peripheral reservoir. The problem of the importance of peripheral versus central reservoirs has received considerable attention (16, 18, 20, 21) without solution. The capacity of the whole vascular system, and also of the arterial tree alone, has been estimated for dogs (33, 34).

Sollmann & Gilbert (35) have noted that even in excised vessels contraction of the circular coat causes lengthening of the vessel. The lengthening of vessels and increase in tortuosity that accompany rise of blood pressure need not all be due to passive longitudinal stretch.

The question whether the systolic pressure in the aorta is significantly lower than that in the ventricle has been again raised (36). These two values are found to be practically identical and indicate basal systolic and diastolic pressure values in the dog which average 124 and 85 mm. Hg respectively. The tracings shown to prove identity were, however, all obtained during periods of high blood pressure induced by epinephrine to "increase the velocity of blood leaving the heart." The assumption that the velocity of outflow is increased at the time of the greatest peripheral resistance is unwarranted. If velocities are high the pressures cannot be by any means identical.

#### CARDIAC OUTPUT: TOTAL BLOOD FLOW

The rate of blood flow continues to excite interest. The variations of basal flow with age have been statistically analyzed by Lewis (37); the arteriovenous oxygen difference remains the same. A reduction in cardiac output (averaging 38.5 per cent) during standing is confirmed by McMichael (38); such reduction is shown to be associated with peripheral vasoconstriction in the extremities

(39).<sup>3</sup> Recent work (40) has demonstrated that the earlier reports of Grollman that there is little or no reduction of cardiac output on standing can be confirmed, provided that the subjects be adapted to exposure to hot weather. Grollman's data were obtained mainly under these conditions. An increased blood volume appears to be associated with this altered reaction to posture. The low metabolism of myxedema is associated with a still greater reduction in cardiac output, so that the arteriovenous oxygen difference is increased (41). These changes are contrasted with those of hyperthyroidism and a cardiac stimulating factor in the thyroid secretion is assumed. Such an assumption is unwarranted. Changes in heat production demand adaptation of heat loss. The skin circulation must be changed, and to produce the necessary alterations in surface temperature the changes must be great. Consequently there is no simple linear relation between blood flow and oxygen consumption. Alterations in cardiac output with meals and with changes in environmental temperature are reported (20, 21). A reduction of cardiac output averaging 41 per cent following severe operations has been observed, with recovery of normal values only after one to four days (42). Such a reduction is not seen as the result of one hour's etherization without operation.

#### CAPILLARIES AND VEINS

Study of the amount of blood in peripheral vessels in mice killed under different conditions has been continued by Sjöstrand (43). The blood contained in such vessels after laparotomy under anaesthesia is much greater than that observed when small mice are suddenly frozen in liquid air. Study of the capillaries in heart and gastrocnemius muscle in animals killed by a large dose of histamine demonstrates that the number of capillaries per cubic millimeter is larger in animals trained to heavy exercise for two weeks. The increase is greater in young animals (44). Capillaries in the skin have been stated not to show intermittence; this is denied (45).

Studies of blood flow in a chamber in the rabbit's ear have been made during anaphylactic shock (46); contraction obliterates the

<sup>3</sup> Constriction was demonstrated by a fall in surface temperature. Under somewhat different conditions later reports indicate a rise in temperature. See Roth, G. M., Marvin, M. D. W., and Sheard, C., *Am. J. Physiol.*, **124**, 161 (1938).

lumen of the arterioles and stops blood flow, but there is no contraction of capillaries or venules. Additional data on the permeability of capillaries by Landis' direct method have been reported by Wind (47). Capillary permeability in inflammation has been studied by Menkin (48) by injection of trypan blue. Inflammatory exudates (49) contain a substance which increases capillary permeability. This substance is not histamine, but probably a polypeptid.

Direct measurement of venous pressure in man has been made with optical records of changes produced by respiration, change of posture and temperature (50). In the supine position pressures in different veins are equal to the height of the manubrium sterni above them; the blood may be considered as just getting over the dam. In the erect position the pressure in the long saphenous vein is adequate to raise blood to the third intercostal space and in the median basilic to the first rib. Respiratory movements affect the pressures but negative waves at sub-atmospheric levels are not transmitted in a retrograde direction owing to collapse of the veins (50). Exercise raises venous pressure (51); the maximum increase is of the order of 90 per cent with work at 8000 ft.-lbs. per minute. After exercise the pressure gradually returns to normal but after heavy work this change may take over twenty minutes.

The effect of venous pressure on tissue fluid pressure has received extensive study (52, 53). Wells and his coworkers (53) have distinguished between the tissue fluid pressures that develop in dermal, subdermal, and muscular tissues. The two former tissues offer little resistance to collection of fluid, and rise in venous pressure does not greatly increase tissue fluid pressure, but conditions are very different in muscles with a tense fascial sheath. In the dermis, subdermis and muscle normal values are of the order of 7, 3 and 5 cm. of water; with venous congestion these may rise to 10, 15 and 50 cm. respectively. The marked pressure rise in the muscle hinders fluid transudation; tissue fluid pressure is always below venous pressure.

#### NERVOUS CONTROL OF THE CIRCULATION AND LOCAL EFFECTS OF METABOLITES

For convenience of description I would like to distinguish between intrinsic reflexes concerned with the regulation of the circulation in relation to itself, and extrinsic reflexes concerned with

regulation in regard to the needs of the tissues. The intrinsic reflexes prove to be more complex than they have been previously represented. It has been shown by Heymans and his coworkers (54) that, even in the spinal dog, vasomotor reactions in both the spleen and leg may develop to changes in pressure in the aorta, though both carotid sinus and aortic arch reflexes are excluded. The reactions depend mostly, but not entirely, on pressure changes in the areas supplied by the coeliac axis and superior mesenteric arteries. Impulses derived from the Pacinian corpuscles described by Gammon & Bronk (54a) are considered involved. The presence of a residual response of this type, even after exclusion of the abdominal viscera, is confirmed (55).

By separate artificial perfusion of the systemic and pulmonary circulations Daly and his coworkers (56) find that an increase in pulmonary blood flow causes a fall in systemic pressure. Obstruction to the outflow from the left atrium is effective but not a rise in pulmonary arterial pressure. Nonidez (57) described receptor endings not only in the venae cavae but also in the pulmonary veins. These may be involved. Periodic fluctuations in pressure in the pulmonary system unaccompanied by systemic changes could also be demonstrated, so that the resistance of the pulmonary vessels is not constant (56). The receptor innervation of the aortic body was described by Nonidez (58); more recently the nerve fibers to this body have been shown by Comroe (59) to pass, partly at least, by the recurrent laryngeal nerves. The body is demonstrated to function as a chemo-receptor. Effects of sodium cyanide and the like on the circulation as evidenced by blood pressure changes are almost entirely dependent on this body and only slightly on the carotid body (59). Bernthal (60) on the other hand finds the carotid bodies quite effective in producing vascular changes. They are sensitive not only to oxygen lack but also to changes in carbon dioxide tension. Bernthal's method of limb plethysmography should be more sensitive in detecting such reactions than blood pressure measurements. His experiments, however, do not preclude the possibility that the aortic body may be the more sensitive. The vascular responses to distension of the carotid sinus have been investigated by Winder (61). The sinus effects on blood pressure are less modified by impulses from the aortic area than are those on the pulse rate.

The vasomotor response to oxygen lack is reversed from a pres-



sor to a depressor reaction in dogs by denervation of the carotid sinuses and section of the vagi (62). The pressure rise is considered as entirely due to stimulation of chemo-receptors, while the center itself is merely depressed by oxygen lack. This cannot be the complete story, for the classical experiments of Mathison (62a) demonstrated that a marked rise of blood pressure might be induced by simple oxygen lack in a decapitated cat. Here any involvement of nerves from the aortic and carotid bodies can be excluded. Oxygen lack decreases the pressor reflexes resulting from varying distension of the carotid sinus, as evidenced by postural hypotension (63). Postural hypotension has also been described as a symptom of the late effects of carotid sinus denervation in man (64). Changes in blood pressure produced by stimulation of the carotid sinus are not accompanied by changes in blood flow in the kidney as they are in other organs (65). Denervation of the carotid sinuses and aorta in rabbits is described as inducing not only hypertension but also new formation of red cells (66). A hypothetical brain center is invoked. Anoxia in the bone marrow from involvement of its blood supply in general vasoconstriction supplies an alternative and more probable explanation. Oxygen lack produced by carbon monoxide mixed with oxygen, so that the oxygen content of arterial blood is low but tension high, does not produce a pressor response (67). Lowered oxygen tension appears to be the essential stimulus.

Whether peripheral intrinsic mechanisms are concerned with regulation of the circulation in the extremities is uncertain but complicated mechanisms exist which regulate the circulation in relation to the metabolic needs of the tissues. Among these are local dilatation effects due to the direct action of metabolites on vessels, axone reflexes causing dilatation in neighboring areas, as well as reflex vasoconstriction in other areas. True vascular reflexes originating in obscure peripheral sensory impulses undoubtedly exist. It has been shown by Doupe, Robertson & Carmichael (68) that vasoconstriction is more readily induced in the vessels of the toes than in the fingers; "spontaneous" waves of constriction may develop. Responses are not seen after sympathectomy (the sympathetic represents the motor path) nor spontaneous waves in the presence of cauda equina lesions which block sensory impulses from the legs. A continuous bombardment of impulses from the legs seems essential to maintain the high degree of reflex sensitivity, in which central mechanisms must be involved.

The muscles may give rise to impulses which may induce great changes in the circulation. Alam & Smirk have demonstrated a pressor change of 10 to 90 mm. Hg (69), and increase in pulse rate of 3 to 20 beats per min. (70), when muscles are exercised while the circulation is arrested by a Riva Rocci cuff. The change is reflex, for it is maintained after exercise if the circulatory arrest is continued. It disappears when the circulation is released. The reactions often precede any accompanying pain. The pressure change varies with the work done and the response may be obtained from small muscles. The pulse rate change is usually only obtained from the larger muscle groups. A similar type of response may be concerned in the experiments of Asmussen, Christensen & Nielsen (71). The obstruction of the circulation to both legs at rest by Riva Rocci cuffs causes no change in blood pressure in the arm. Compensation is effected. If this obstruction is long maintained, on release there is a fall of pressure and increase in pulse rate. If this release lasts only 20 sec. on reobstruction of the circulation the pressure is raised some 30 mm. and the pulse rate is slowed some 10 to 15 beats. The changes in pulse rate are considered as secondary to those in blood pressure.

Simple axone reflexes constitute one part of the multiple responses to local collections of metabolites as well as to reactions to temperature stimuli. Wybauw (72, 73, 74) describes vasodilatation in the digits of a cat or the ear of a rabbit on electrical stimulation of the posterior roots even after sympathectomy. The response is also present nineteen days after section of the roots. The changes are prolonged by eserine. A perfusate of the pad of cat's foot obtained during such stimulation contains a substance showing the properties of acetylcholine. Dilatations to extreme cold and heat involve these mechanisms (74). Axone reflexes are involved in the spread of local vasodilatation during activity and affect also large arterial trunks such as the main femoral artery (75, 76). As was pointed out by Rein at the Physiological Congress (1938), one must be cautious in one's interpretation of widespread responses. A contraction of the spleen need not imply a direct effect of the primary reflex, for local dilatation may secondarily induce readjustments through the intrinsic buffer mechanisms.

Many metabolites may be concerned not only in the production of the direct dilatation of vessels but also in the initiation of reflex or axone reflex changes. Among these must be considered not

only carbon dioxide, but also many other products. Fleisch & Weger (77) have shown that phosphorization of substances such as creatin often intensifies, or even creates, a capacity to produce vasodilatation. One of the most powerful of these vasodilator substances is adenosinetriphosphate, which can induce dilatation in molecular concentrations of  $0.25 \times 10^{-6}$ , a dilution approximating that effective with acetylcholine ( $1 \times 10^{-8}$ ). A chemical vasodilator is also liberated in hemolysis of red corpuscles (78, 79). This also may be some adenosin phosphate compound. Fleisch & Weger (80) emphasize that under normal conditions of blood flow vasodilator substances cannot be detected in the venous blood issuing from an active area; it is a phenomenon limited to an impeded circulation. Similar compounds and their axone reflex effects are likely to be involved in setting up of collateral circulatory paths after ligation of large arterial trunks (81).

Rein and his coworkers (82, 83, 84) have obtained evidence that, when the circulation to a muscle is reduced reflexly, the metabolism of the muscle is also reduced. If the blood flow to a muscle stimulated electrically be mechanically impeded, the muscle contractions are weakened by fatigue, and release of the obstruction is followed by a reactive hyperemia. On the other hand if the blood flow to the active muscle is reduced through the effect of an excess of carbon dioxide acting on the vasomotor mechanisms, reduction of the muscle contractions is not due to fatigue but to reflex inhibition. Not only is contraction reduced, but also the length of the relaxed muscle is greater. When the reflex vasoconstriction ceases, no reactive hyperemia is seen (82). If the oxygen usage of muscle is estimated from blood flow measurements and records of the oxygenation of arterial and venous blood, mechanical interference with the blood flow for one to three minutes causes no reduction in oxygen consumption; any initial deficit is made up during the subsequent reactive hyperemia (83). On the other hand if the vessels are constricted through reflex effects from the carotid sinus, both reduced oxygen consumption and reduced heat production can be demonstrated (84). Gremels & Zinnitz (85) have demonstrated on spinal cats that peripheral vagal stimulation as well as injection of acetylcholine and small doses of epinephrine reduce oxygen consumption. Rein believes such changes may be related to the phenomena he has described.

A reflex vascular response of considerable interest is that de-

scribed by Irving (86) in relation to respiration. He noted in diving mammals, such as the beaver, a reflex decrease in blood flow to the muscles with increase in that to the brain on inflation of the lungs. The reflex was one apparently associated with the arrest of respiration in diving. The same adjustment to lung inflation and respiratory arrest has now been demonstrated in cats, dogs and rabbits. It is not dependent on the production of apnoea and may be seen on cessation of artificial respiration in a curarised animal. The decrease in flow is of nervous origin and is absent in a denervated area. Removal of the carotid sinuses accentuates the response. While cessation of respiration is a consistent factor, increase in carbon dioxide tension is probably too slow to account for the reflex change which develops in twelve to twenty seconds. Probably the changes are related to the peripheral constriction demonstrated in man by Carmichael (86a) and his associates to accompany a deep inspiration.

The question whether variations in vascular tone can be induced reflexly in the absence of the sympathetic, except in the form of axone reflex responses is still unsettled. Thomas & Brooks (87) could find no evidence of such changes in response to stimulation of carotid sinus in sympathectomised cats. Slight increases in pressure resulting from occlusion of the carotid arteries could be explained on simple physical grounds. Heymans and his collaborators (88) have reinvestigated the subject and confirm their own earlier positive results in decerebrated cats. In cats occlusion of the carotid arteries might cause an increase in pressure of 90 mm. Hg (to a level of 220 mm.) in the absence of all sympathetic fibers as evidenced not only by post-mortem examination but also by the absence of pressor responses to sciatic stimulation. The response may be small and indefinite if the initial blood pressure is low, or if the response be depressed by anesthetics. They believe the discrepancies in the literature to depend on these factors.

#### CHEMICAL FACTORS AFFECTING THE CIRCULATION AND HYPERTENSION

It is not easy to differentiate between the local effects of metabolites, and the effects of chemical substances such as epinephrine which can act at a distance. The former have already been briefly discussed; some of these may under certain conditions reach sufficient concentrations in the blood to produce general effects. Epineph-

phrine has long been recognized to exert different effects in active organs, particularly in the presence of local acidity. Doses of epinephrine which induce vasoconstriction in the vessels of a resting muscle fail to do so during prolonged muscular work (93). This change depends on the chemical conditions, for epinephrine constriction is induced in stimulated but curarised muscle, and also in muscle during and after a brief tetanus. The chemical changes of activity do not prevent vasoconstriction to electrical stimulation of vasoconstrictor nerves (93), though the threshold for such vasoconstriction is raised during impaired circulation to a muscle produced mechanically as well as during the reactive hyperemia which follows such mechanical obstruction (83). Vasodilators such as acetylcholine, histamine, and adenylic acid still act on the vessels of actively contracting muscle (94), but not on those of the skin dilated by exposure to heat (95). Epinephrine constriction is still obtainable in such skin vessels. The underlying mechanisms of such dilatations must be different.

The time relations of reactions to intra-arterial injections of epinephrine have been investigated by Roome (96) by the utilization of a recording stromuhr of Ludwig's type in the femoral artery. The reaction to small doses (0.025 to 2  $\mu$ g) of epinephrine varies and may be constriction only, dilatation followed by constriction, or constriction with a later superimposed dilatation. Simple constriction is seen particularly when there is a background of general vasodilatation, as after acute denervation. Dilatation is seen early when the initial blood flow is rapid, and late when this is slow. The results are interpreted on the following hypothesis. Epinephrine produces simple constriction of arterioles, but after a lag which depends on the time required for diffusion through a thick wall. Epinephrine produces simple dilatation of capillaries after a lag which varies with the rate of flow and the consequent rate of transmission of epinephrine to the capillaries.

Considerable doubt exists whether substances such as acetylcholine and sympathin normally exert any influence on the circulation except at their points of liberation. The substance norepinephrine ( $\beta$ -3,4-dihydroxy-phenyl- $\beta$ -hydroethylamine) has been investigated by Greer *et al.* (100) and evidence advanced suggesting that *L*-norepinephrine may be identical with sympathin E and that *L*-epinephrine may be identical with sympathin I.

Only a few of the numerous papers that have appeared on ex-

perimental hypertension from interference with the renal circulation can be cited (102, 103, 104). The evidence that the mechanism is a humoral one is overwhelming (105, 106, 107, 108), though hypertensions with a reflex factor (109) can apparently occur. The high pressure in the cephalic part of the arterial tree in coarctation of the aorta is also of renal origin (110, 111). The adrenals have no primary relationship to the condition, except as they affect the circulation in general (112, 113, 114). Two hypertensive substances can be extracted from renal tissue and are obtained in larger amounts from ischemic kidneys (115, 116, 117, 118, 119). Attempts to demonstrate variations in pressor substances in the blood or urine as the result of renal ischemia have failed (120, 121). Cure of experimental hypertension has been attained through establishing a collateral circulation to the kidney by contact of the decapsulated kidney with the great omentum (122). The relation of endocrine glands to essential hypertension is discussed in a series of papers by Westphal & Sievert (123 to 129). They view essential hypertension as due to a derangement of the pituitary and adrenal glands and claim that a pressor substance is found in ultra-filtrates of the blood of such patients. Few would deny that such disturbances may cause hypertension, few would agree that they are commonly involved. Excessive doses of vitamin D<sub>2</sub> induce arteriolar hypertrophy particularly in the kidney (130) and cause hypertension; the thyroid is stimulated (131); after thyroparathyroidectomy the renal arterioles are not affected but the large arteries become sclerotic. After thyroidectomy with preservation of the parathyroids, the large vessel changes are greater (130). Thyrotropic pituitary hormone increases the atherosclerotic changes produced in rabbits by cholesterol (132). These changes are prevented by "lipocaic" (133) but not by choline feeding (134, 135).

#### SURGICAL SHOCK

This subject cannot receive detailed treatment here. Reduction in blood volume is confirmed and is associated with increased interstitial fluid (136); cardiac output is reduced (42). Blood histamine is only slightly increased in shock even in the blood leaving a traumatized area (137). Total sympathectomy renders dogs more susceptible to hemorrhage, but they do not develop the vicious circle symptoms of shock (138); the absence of shock is attributed to the absence of compensatory vasoconstriction and consequent

capillary changes from anoxemia. A study of circulatory failure in adrenal insufficiency (139) and its comparison with shock due to epinephrine injections (140) lead Swingle *et al.* to the conclusion that capillary tone and consequently the volume of the capillary system are controlled by the adrenal cortical hormone. The effects of increased vascular bed or decreased blood volume, changes in filtration pressure or colloid osmotic pressure are hard to disentangle. Repetition of crossed circulation experiments on traumatized limbs failed to confirm the development of shock of nervous as well as of mechanical or chemical origin (loss of fluid into the damaged tissue) that had been described. With nembutal anaesthesia only the latter was found (141).



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DEPARTMENT OF PHYSIOLOGY  
UNIVERSITY OF PENNSYLVANIA  
PHILADELPHIA, PENNSYLVANIA

## RESPIRATION AND ITS ADJUSTMENTS\*

BY ROBERT GESELL

*Department of Physiology, University of Michigan  
Ann Arbor, Michigan*

If ever there was a conviction firmly intrenched in physiology, it was the monopoly of the chemical control of breathing by the respiratory center. Based as it was on circumstantial evidence, it proved to be one of physiology's outstanding creeds. To be sure, we had the crossed circulation experiments of Fredericq (1), beautiful and convincing, seeming to establish the central chemical control of breathing. We had too the perfusion<sup>10</sup> experiments of Winterstein (2) and Hooker, Wilson & Connett (3). But we need only review the evidence in a critical way to realize that no one had actually demonstrated that either excess of carbon dioxide or lack of oxygen, restricted solely to the medulla, is capable of augmenting pulmonary ventilation. Such central stimulation was simply taken for granted. Indirect evidence was accepted as direct proof. Statements going unchallenged were eventually accepted as facts. Physiologists knew that the chemical control of breathing resided solely in the respiratory center, for a minute structure lying deep in the carotids had been overlooked. Rosenthal, Hermann, Pflüger, Markwald, Geppart, Zuntz, Haldane, Barcroft, Miescher, Priestley, Douglas, Luciani, Winterstein, Hooker, Hasselbalch, Lundsgaard, Loevenhart, Gasser and many others and such august bodies as the British Hemoglobin Committee were not in doubt. We all were sure. The really pertinent question seemed to be how does the respiratory center manage its control. For example, Loevenhart (4), impressed with the shortness of the latent period of the respiratory center to the injection of cyanide, states, "This extremely rapid action precludes all possibility of explaining the stimulus as due to excess of CO<sub>2</sub> or accumulation of acid products. It proves beyond peradventure that the cells (of the medulla) respond with stimulation to a decrease in their own oxidation."

It was a shaky foundation upon which all of us worked. So when Heymans, *et al.*, (5) produced hyperpnea by a lack of oxygen or an excess of carbon dioxide confined to the aortic (1924-27) and

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carotid chemoceptors (1930-32), he gave to us a new outlook on respiration for which physiology is deeply indebted. Central chemical control which had been accepted on faith, was now on the defensive. In the confusion, many adopted new faiths which carried them too far, for luck was with the majority. Both central and peripheral chemical control were proven to be extremely important, and the principles of chemical control developed on the respiratory mechanism as a whole, seem now to be applicable for its parts.

#### THE TWO MAIN THREATS TO THE SUPPLY OF OXIDATIVE ENERGY

In coordinating the factors of respiration, one must mark well their sole objective—the provision of energy for the running of the living machine, Nature's oldest and most pressing problem which has shaped our bodies as they are today. Our maintenance of energy, dependent primarily upon oxidations, just as in the primitive amoeba, demands a supply of oxygen proportionate to the energy requirements of the machine. Since the accumulation of carbon dioxide leads to increasing hydrogen-ion concentration which impairs oxidation, and since the formation of carbon dioxide is proportionate to the rate of oxidations, elimination of carbon dioxide must have proportionate attention.

In times of stress, when oxygen is scarce, non-oxidative energy momentarily substitutes for energy provided by the way of oxidations. But in that process lactic acid, formed in greater abundance than carbonic acid, combining with sodium bicarbonate liberates carbonic acid and turns the protoplasm more acid. Because excess of acid as well as lack of oxygen reduces and imperils oxidative energy, both are usable as warning signals in combatting energy shortage, from the protozoan up to the current mammal.

#### COORDINATED CONTROL OF VENTILATION, CIRCULATION AND ENERGY SUPPLY

The all-inclusive control of energy is a most intricate combination of physical and chemical phenomena. But the rapid adjustments meeting the fleeting emergencies of the day, resting primarily on a dual gaseous exchange, are dependent mainly upon pulmonary ventilation and the circulation of the blood. Greater



pulmonary ventilation by increasing the oxygen loading of the blood and carbon dioxide unloading reduce the need of and the stimulus for energy. Greater volume flow of blood by improving the transportation of oxygen to the tissues and carbon dioxide to the alveolar spaces likewise reduces the need of and stimulus for energy. Perfectly useless, one without the other, they work in closest cooperation through the aid of similar and common mechanisms of control.

These mechanisms, physical and chemical, control the flow of blood and air. The respiratory and circulatory centers and their outlying chemoceptors respond to chemical changes in their immediate environment. Stimulation of the chemoceptors produces simultaneous effects on ventilation and circulation, but the manner in which this multiple action is accomplished is not yet known. Common reception of a chemical change at a single chemoceptor and multiplicity of central connections of that receptor with the circulatory and respiratory centers seems the simplest mechanism allowing individual receptors to simultaneously impress their influence upon breathing, pulse rate, blood pressure and volume flow of blood. With such an arrangement, quantitative distribution of the central connections would determine the relative intensity of the effects. Thus a common basic reaction of all peripherally lying chemoceptors to chemical changes could meet in a simple way the rigid requirements of coordination of ventilation and circulation.

Acting solely by themselves, the chemical mechanisms, central and peripheral, can function in a most efficient manner for they can sense directly the warnings of hampered oxidations. Yet superimposed upon these mechanisms are complementary systems of control, driven solely by physical forces. Stretching of the carotid sinus and aortic arch inhibit respiratory and circulatory muscles. Stretching of the proprioceptive endings of the lungs modify breathing and circulation. Unable directly to sense changing oxidations, but quick to feel a drop in pressure which would curtail oxidations, the vascular proprioceptors forewarn and forestall a chemical change, which through delays of direct chemical control might assume much larger proportions. Quick to feel a volume change of the lungs, the pulmonary proprioceptors are as prompt to modify circulation and ventilation in their own way.

PRIMARY MEANS OF COMBATING THREATS TO  
OXIDATIVE ENERGY SUPPLY

A logical analysis of respiratory control must explain the major responses of the body to the two primary conditions of oxidative stress; namely, lack of oxygen and excess of metabolic acids. It is now well established that gaseous mixtures low in oxygen, administered by uniform artificial ventilation, diminish oxygen consumption and oxidative energy, despite increased volume flow of blood. Non-oxidative energy, arising from the transformation of glycogen to lactic acid, fails to meet the deficit and the sum total of energy is curtailed. Due to the rapid formation of lactic acid the sum total of acid formation exceeds the normal. This, combined with a reduction of carbon dioxide eliminated by the lungs, leads to acid accumulation in the body. The resulting increase in tissue acidity further impairs oxidation and thereby sets in motion a dangerous vicious cycle [Gesell, Krueger, Gorham & Bernthal (6)]. Increased circulation by itself is unable to cope with anoxemia.

When, however, a normal increase in ventilation is allowed to combat the shortage of oxygen in conjunction with the circulatory changes, the picture reverses [Gesell, Krueger, Nicholson, Brassfield & Pelecovich (7)]. Excessive reduction of alveolar oxygen is avoided. The better maintenance of oxygen tension assures greater oxidations and a greater liberation of oxidative energy and a corresponding reduction in the formation of lactic acid. Carbon dioxide, eliminated far in excess of normal, decreases the hydrogen ion concentration of the tissues as a whole below the usual levels. This decrease in tissue hydrogen-ion concentration coupled with other chemical adjustments accomplishes a greater utilization of oxygen by the tissues and an actual increase in oxidative energy, even during the period of oxygen want. This, added to the augmented oxidative energy of recovery, results in a gross increase in energy over that of a corresponding period of ordinary basal conditions.

Computations indicate that the excess energy spent during anoxia and recovery is more than adequate to meet at least five important adjustments: (1) extra mechanical energy for augmented pulmonary ventilation; (2) extra mechanical energy for augmented flow of blood; (3) extra thermal energy for the warming

of the increased tidal air; (4) extra thermal energy for the saturation of the increased tidal air with water vapor; (5) increased energy for the liberation of carbon dioxide from the blood into the alveolar air. It was, therefore, suggested that the free balance of energy remaining after these deductions might be employed to establish special chemical adjustments to promote freer oxidations and to repair possible cellular damage resulting from anoxia. The insidious damage which anoxia may produce and the need of its repair are well described in Dill's recent book on "Life, Heat and Altitude" (8). We may therefore conclude that in times of oxygen stress the body will call into play costly energy consuming processes to maintain the needed energy at par and to minimize the bodily harm which might otherwise occur.

Cyanide, believed to produce its physiological effects like oxygen lack through interference with oxidations [Geppart (9); Euler & Liljestrant (58)], produces similar results to those of simple anoxemia [Gesell, Krueger, Nicholson, Brassfield & Pelecovich (11)]. Pelecovich (12) found increased oxidation in 85% of her observations. Marshall & Rosenfeld (13) noted protracted hyperpnea without reduction in oxidations upon injecting pyruvic acid cyanhydrin which liberates cyanide slowly in the body. Simple hypercapnia produced by administration of carbon dioxide increases the hydrogen-ion concentration of blood and tissues and lowers oxidations [Gesell, Krueger, Gorham & Bernthal (98)]. Hypercapnia even with great excess of oxygen pressure interferes with oxidations [Bean (14)]. Hyperpnea and increased flow of blood tend to correct this interference. Our problem is to learn the mechanism by which such corrections are accomplished.

The comparison of the effects of anoxemia and cyanidemia and of hypercapnia permit of two significant conclusions related to the chemical mode of stimulation of breathing: (1) other things remaining constant, oxygen lack leads to decreased oxidations and a consequent increased intracellular acidity; (2) other things remaining constant, carbon dioxide excess leads to increased intracellular acidity and a consequent decrease in oxidations. The hyperpnea of either anoxia or hypercapnia could then be due to either impaired oxidation or increased acidity or both, at the chemosensitive structures. This subject, one of long standing discussion, is considered in detail in our older review, but the more recent observations by Pelecovich (12) are relevant. Comparing

the effects of intravenous injection of cyanide and hydrochloric acid, she found the increased ventilation per unit impairment of oxidation to be approximately 2.5 times greater with injection of hydrochloric acid than with sodium cyanide. The greater importance of the hydrogen-ion concentration is thereby indicated.

Attempts to relate changes in pulmonary ventilation to corresponding changes in hydrogen ion concentration of the blood, however, continue to be as futile as before the discovery of chemoceptor function. We need only recall the outstanding contradictions [Gesell (15, 16)]: hyperpnea and decreased arterial hydrogen-ion concentration of anoxemia; hyperpnea and no change in arterial hydrogen-ion concentration of cyanidemia during constant artificial ventilation; the opposite effects of sodium carbonate and sodium bicarbonate on breathing, although both produce the same changes in blood hydrogen-ion concentration. Only by relating intracellular hydrogen-ion concentration to pulmonary ventilation does contradiction seem to disappear.<sup>1</sup>

#### RESPONSE OF CENTRAL AND PERIPHERAL CHEMOSENSITIVE MECHANISMS TO BICARBONATE BUFFER CHANGES

With the discovery of the carotid and aortic chemoceptive function, the problem now assumes a broadened interest, for with appropriate isolation, localized chemical action at the center or at the carotid body may be determined. Such studies show increased ventilation from localized central action of carbon dioxide and decreased breathing from local hypocapnia. Intravenous or intra-

<sup>1</sup> Advantageous as it is to establish the preeminence of any single factor, it seems unwise, as we have suggested before (15) to accept hydrogen-ion concentration as the sole means of chemical control. The need of caution on this point has been sufficiently stressed by Henderson (17). We believe there is now justification to consider the relation of changing hydrogen-ion concentration to all phases of respiratory excitation if at the same time sight is not lost of the invariable accompaniment of changing oxidations. So too, is it essential to realize that the liberation and accumulation of acid are but steps on the way to an end. How far they are removed from that end is still unknown. Central and peripheral excitation of breathing by acetylcholine and potentiation by eserine and prostigmine [Anitchkov *et al.* (18); Heymans *et al.* (19, 20); Chang *et al.* (21); Worzniak & Gesell (22); and Miller (23)] suggests a possible control through the release and protection of acetylcholine by liberated acid [Winder (25)]. Other fundamental membrane phenomena closely related to excitation such as polarization and depolarization currents and the mobilization of ions will call for the closest study.

vertebral arterial injection of sodium carbonate and sodium bicarbonate after complete chemoceptor denervation, decrease and increase ventilation respectively (Gesell, unpublished). Though turning the blood more alkaline, sodium bicarbonate turns the cerebrospinal fluid more acid [Gesell & Hertzman (26)], thus associating the accompanying hyperpnea with a probable increased acidity of the respiratory center. Conversely, carbonate which turns the cerebrospinal fluid more alkaline associates the accompanying apnea with a decreased hydrogen-ion concentration of the center.

It is now equally well established that a rise or fall in carbon dioxide pressure of the blood circulating through the carotid body has the same effects upon respiration as described for the center [Heymans *et al.*, 1930-32 (5); see also 28, 29, 30, 27, 70, 40, 31, 49, 10] and through the careful studies of Bernthal (31) demonstrating and analyzing the opposite effects of sodium carbonate and sodium bicarbonate, we may postulate as before (15, 16) that changes in intracellular acidity resulting from simple changes in the carbonate buffer system, whether acting at the center or at the chemoceptor, are the predominant chemical factors of control.

The effects of bicarbonate and carbonate upon circulation are like those upon ventilation. Administration of bicarbonate limited to the circulatory centers (Gesell, unpublished) or to their subservient chemoceptors [Bernthal (31)] produces vasoconstriction and a rise in pressure, and similarly restricted action of carbonate produces a fall in pressure. Excessive and inadequate artificial ventilation after complete chemoceptor denervation respectively lower and raise blood pressure even more than in the intact animal [Heymans & Bouckaert (32)]. Such findings speak for a common underlying scheme of control of the circulatory and ventilatory systems for both the central and peripheral regulatory mechanisms.

#### THE ACID METABOLISM OF THE CENTRAL AND PERIPHERAL CHEMOSENSITIVE MECHANISMS

The respiratory and circulatory centers and their outlying chemoceptors, all demonstrably sensitive to hydrogen-ion concentration, should logically be capable of detecting not only changes in exogenous acid arriving by the blood, but changes in their own production of carbonic and lactic acid as well. By virtue of their

own acid metabolism, aerobic and anaerobic, they should feel their own oxidative needs and thereby automatically provide suitable oxidative conditions for the body as a whole. Such an hypothesis unites center and periphery in a common response to the two primary threats to oxidation—oxygen lack and carbon dioxide excess.

This then may be considered as an extension of our original hypothesis (15, 16) which included the respiratory center alone. Inasmuch as the carotid body is a relatively simple structure, devoid of complicated peripheral connections, and capable of unquestionable circulatory isolation, it is susceptible to precise investigation and accordingly has yielded most significant information. To establish a fundamental principle with relative certainty under simple conditions allows clearer use of that principle under more involved situations. Therefore, the role of metabolism in chemoregulation, though initially applied to the respiratory center, is first analyzed for the carotid body.

Conforming with the postulated role of metabolism, a local rise of temperature of the carotid body designed to augment the metabolic production of acid (carbonic and lactic) increases pulmonary ventilation and constricts the blood vessels [Bernthal & Weeks (33)]. Conversely, a blockage of the nutrient artery designed to hamper the transport of oxygen to and acid from the carotid body stimulates breathing and vasoconstriction [Winder, Bernthal & Weeks (34)]. Anoxemia and cyanidemia which are known to cause an accumulation of lactic acid produce the same effects through the chemoreceptors [Pagano (35); Heymans *et al.* 1924-1927 and 1930-1932 (5); see also (38, 39, 28, 43, 44, 55, 57, 36, 37, 59, 45, 42, 56, 69, 70, 46, 47, 51, 10, 48, 50, 40, 52, 31, 49)]. Direct experiments indicate that such an accumulation of acids resulting from glycolysis is an essential step in the production of anoxic hyperpnea for when anaerobic glycolysis is prevented by localized poisoning with mono-iodoacetic acid increased breathing is missing though hyperpnea is still produced by localized hypercapnia [Winder (40)].

The rapid accumulation of lactic acid demonstrated by McGinty & Gesell (41) in the brain of the decapitated dog gave strong support to the theory of central metabolic stimulation by endogenous acid. But actual experiments on the central action of oxygen lack have on the whole revealed variable results. One

group of papers emphasizes a non-stimulant or depressant action [Schmidt (28); Selladurai & Wright (43); Beyne, Gautrelet & Halpern (44); Wright (45, 46); Jongbloed (47); Smyth (48); Comroe & Schmidt (49)]. Another group describes occasional stimulation, readily subject to depression [Heymans *et al.*, see (5); Gesell & Moyer (50); Marshall & Rosenfeld (13, 51); Dautrebande (52, 53)]. Still another group finds definite excitatory reaction in chronically denervated, unanesthetized animals, of a nature to afford satisfactory altitude acclimatization [Decharneux (54); Dautrebande (52, 53)]. Under certain conditions, Marshall & Rosenfeld (51) found that high oxygen administration depressed respiration in similarly denervated animals. These numerous positively stated evidences of stimulation assume greater significance on considering that five of the eight papers of the first (negative) group allow, either by tracings, charts or statement, of the possibility of occasional stimulation. We are convinced that excitation as well as depression occurs and that the change in breathing produced by central action is a resultant of excitation and depression.

Although the action of cyanide on tissues is presumably equivalent to that of anoxia, the central action of cyanide is more definitely excitatory than that of anoxemia (tentative explanation later). In our hands, cyanide almost invariably gave evidence of excitation after chemoceptor denervation whether applied to the floor of the fourth ventricle, injected into the vertebral artery or injected intravenously. Heymans *et al.* (5) who were the first to demonstrate strong peripheral action, describe only occasional central stimulation. A late, long-lasting central excitation, sometimes of remarkable force, was a common experience of Winder, Winder & Gesell (55) and was also observed by Wright (56), and Marshall & Rosenfeld (13). With suitable dosage an earlier, more evanescent phase of excitation is frequently seen, which may also be of considerable magnitude [Winder, Winder & Gesell (55); Camus, Bénard & Merklen (57); Marshall & Rosenfeld (13)]. Excessive dosage may displace or cut short the early excitation with depression [Winder, Winder & Gesell (55); Camus, Bénard & Merklen (57)], which in turn is commonly followed by a late prolonged stimulation [Winder, Winder & Gesell (55)]. The late, prolonged hyperpnea of cyanidemia in intact [von Euler & Liljestrand (58)] or incompletely denervated animals [Gemmell, Geiling & Reeves (59)] is presumably of the same central action. The



central threshold dose of cyanide in acute experiments, however, seems considerably higher than the peripheral threshold and the response less robust, indicating that the finer regulation against impaired oxidations under those conditions comes through the chemoceptors.

Due consideration of current evidence points to a mechanism available for central chemical control against anoxia even barring Decharneux's (54), and Dautrebande's (52, 53) experiments because of possible nerve regeneration. But whether such a mechanism comes into play in the intact animal has not yet been demonstrated. Such central chemical control as exists we believe is most probably dependent upon a local accumulation of acid, as demonstrated for the carotid body. It is thus pertinent to learn the causes of lowered ventilation which so often accompany impaired oxidations.

If we understand Dautrebande correctly and if he is correct in his observations, the differences between central and peripheral effects of anoxia, during anaesthesia, are due to a relatively great depression of function at the center and little or no depression at the chemoceptors. Finding perfect acclimatization to high altitudes in dogs deprived of the carotid and aortic chemoceptors (52) and demonstrating markedly augmented breathing with decreasing oxygen pressures in the respired air (53), he concludes that the center, free of the depressing action of anaesthesia, is capable of a sensitive response to oxygen lack.<sup>2</sup>

These most significant conclusions will no doubt stimulate further study, for if they stand, it means that both central and peripheral mechanisms are capable of reacting to the two main threats to oxidation—oxygen lack and carbon dioxide excess—as postulated above. One of the most interesting phases thus remaining in the problem of respiratory control is the determination of

<sup>2</sup> The possibility of an adaptive adjustment so common for nervous mechanisms, in this case a development of inherent potential sensitivity of the center to oxygen lack in the absence of normal peripheral support, has so far as we know not been considered. So firmly is the belief of central depression from anoxia established that a late positive response to oxygen lack in chronically denervated dogs is taken as proof of regeneration of chemosensory fibers. See Jongbloed who concludes "*evidement une régénération des nerfs vaso-regulateurs a eu lieu chez ces animaux.*" Let us hope that Dautrebande will have the opportunity of determining in his dogs whether or not regeneration occurred.

the relative importance of central and peripheral adjustments and the manner in which these mechanisms interact.

It is perhaps significant to compare the effects of impaired oxidation on circulation and ventilation. Acting at the carotid body, cyanide produces peripheral constriction and a rise of blood pressure just as certainly as it does increased ventilation. Confined to the centers during anaesthesia, impaired oxidation, commonly produce a rise in blood pressure. The matter, however, is not so simple. As Euler & Liljestrand (10) have recently shown, central anoxia which produces a rise of pressure in the dog, produces a drop in the cat. The central effects of anoxia seem to hang on a most delicate balance.

The depressing effects of anoxia have caused no end of confusion in arriving at clean cut conclusions, perhaps unnecessarily so. At least we have on other occasions tried to see our way through the difficulties which this aspect of the subject presents. For example [Gesell (15)]: "I have noted every grade of response from strong stimulation of respiration to strong depression with changes in oxygen supply, and have to a certain extent been able to supply the conditions for either response. The failure to fully appreciate the double effects of lack of oxygen may lead to opposite interpretation of results; take, for example, the difficulty of Roberts (60) in eliciting virtually nothing but depression of respiration in hemorrhage, and on ligating the cerebral arteries. Similar procedures in the hands of others lead to diametrically opposite results. Such are the effects of oxygen.

"But what are the effects of carbon dioxide? An increase in alveolar carbon dioxide of 1.5 mm. is sufficient to double respiration (61). The effect is so constant that the method of carbon dioxide administration is employed for quantitatively testing the excitability of the center. Mathison states that when carbon dioxide fails to excite lack of oxygen also fails, but carbon dioxide may excite when lack of oxygen fails. Mellanby (62) found striking differences in response to lack of oxygen and carbon dioxide. 'After the respiratory movements of an animal have been diminished or annulled by carbon monoxide a marked stimulation of the respiratory center can be produced by carbon dioxide.'

"If lack of oxygen and excess of carbon dioxide act in the same way, how are the differences in effects explained? The necessary conclusion would seem to be that carbon dioxide produces stimu-

lation by some effect other than reduction of oxidations. On the other hand, granting that reduced oxidations invariably lead to increased production of acids, why does lack of oxygen so frequently fail to stimulate? This might well be due to the general paralytic effect (63) of oxygen lack on living tissue. We need only assume that lack of oxygen has two effects upon the respiratory center; a harmful effect upon the vegetative function, and an excitative effect upon the activity of the center. Since the activity of the center must undoubtedly be an energy consuming process, it would depend to a large degree on the disturbance of the vegetative function which provides the energy. This agrees very well with the general observations of Loevenhart that the better the condition of the center the more readily is the stage of stimulation demonstrated with lack of oxygen."

A consideration of the carotid body in this connection may seem most inappropriate. In that structure, stimulation of ventilation and vasoconstriction by localized anoxemia or cyanidemia is accepted without question. Comroe & Schmidt describe a toughness of the carotid body which it undoubtedly gives evidence of possessing. The experiments of Winder (40) are therefore of interest. He found that during localized mono-iodoacetic acid poisoning, a superimposed local deprivation of oxygen commonly decreased the amount of air breathed and lowered the blood pressure. This occurred at a stage in the poisoning when the receptors had lost their anoxic excitability, retained their hypercapnic response and were for some reason responding tonically with increased intensity. Under such conditions further excitation is impossible with the onset of anoxia for monoiodoacetic acid suppresses anaerobic acid formation as well as oxidative formation of carbon dioxide. Add to this a probable disorganisation of the machinery of the sense organ and there is sufficient cause for a cessation of its tonic stream of impulses. The result is a decreased breathing which in terms of chemoreceptor function means decreased activity of a mechanism invariably responsive under more normal conditions. With this simple background we may envisage more clearly the possibilities of anoxia at the centers, where excitability is admittedly most labile during oxygen deprivation.

Helpful as the carotid body findings may be from point of analogy of central and peripheral machinery, there is as yet no evidence that peripheral depression contributes to the uncertain

effects of anoxemia in the intact animal. Yet the possibility of exhausted chemoceptors ceasing to fire when subjected to oxygen deprivation might be worthy of study.

Closely related to this question of central anoxic stimulation is the less intense stimulating action of simple anoxemia (*no oxygen*) compared with that of cyanidemia, a finding which holds not only for the center (cf. above) but also for the carotid body (Bernthal, unpublished). With opinion agreeing that cyanidemia produces its effects, like anoxemia, through impaired oxidation, explanation of the lesser stimulating action of anoxemia must be sought along other lines than the direct effects of impaired oxidation. Since cyanidemia maintains the blood in a highly oxidized state, its greater stimulation is tentatively attributable to the lesser carbon dioxide carrying capacity of the blood as compared with the greater carbon dioxide carrying capacity of the highly reduced blood of anoxemia, and to the consequent greater accumulation of acid in the tissues.

#### VOLUME FLOW OF BLOOD THROUGH THE CENTERS

In 1922 Gesell, Capp & Foote (64) called attention to the hyperpnea produced by simple hemorrhage and to the subsequent apnea produced by reinjection of the blood. Ignorant at the time of the important discoveries to come, of the influences of the depressor fibers in the sinus and aortic nerves and of the function of the carotid and aortic bodies, we believed the hyperpnea to be due to a deficient flow of blood, and the subsequent apnea to a sudden acceleration of blood through the respiratory center. Shortly after, we advanced the theory of the rôle of acid metabolism in the control of breathing. For as we thought—if there is no change in the composition of the blood we have left to consider, only the effects of altered flow upon metabolism. Now it is known that a simple rise of pressure in the carotid sinus, acting solely as a physical stimulus, is capable of inhibiting respiration; that a restricted flow of blood through the isolated carotid body produces hyperpnea and a subsequent acceleration apnea, as in the experiments of Gesell, Capp & Foote upon the intact dog.

What then is left of our original suggestion? Perhaps much, perhaps little. That question, now under debate between Heymans & Schmidt, gives promise of being as elusive as the effects of simple

anoxia on the centers, for the bearing of anaesthesia and of oxygen lack are similar under both conditions. Heymans and his associates, who revealed the shortcomings of the conclusions of Gesell, have repeatedly failed to find increased ventilation from decreased flow of blood through the centers. Schmidt's positive findings must then be weighed against the negative results from Ghent (5). To quote from Schmidt (65): "This conception (of Gesell) was widely adopted, partly because it is so reasonable, partly because it affords a single explanation for experimental facts that are difficult to explain otherwise. Recently it has been challenged, particularly by Heymans who holds that the respiratory center is not sensitive enough to changes in its blood supply to justify the belief that the metabolism of its cells can be a factor in the regulation of its activity except in extreme cases, which are not of physiological interest. We have already alluded to this question. There are reasons for believing that the respiratory center, under circumstances favorable for demonstrating the relationship, is very sensitive to changes in its blood supply . . . and it is not true that such sensitivity exists only when the center is getting an insufficient supply of blood, as some observers have claimed." Confirmatory evidence is supplied by Stella (66) who occasionally found increased ventilation on clamping of the denervated carotid arteries, despite the fact that they carry relatively little blood to the medulla.

#### CHANGES IN TEMPERATURE OF THE VITAL CENTERS

It will be recalled that Bernthal & Weeks showed increased breathing with localized warming of the carotid body. Nicholson & Brezin (67) had attempted similar experiments with the center. They noted the effects on breathing of changing temperature of the medulla accomplished through external application of heat and cold. The results on breathing were too involved to allow the clean cut conclusion of Bernthal & Weeks. Peculiar combinations of changes in rate, depth, and configuration of the respiratory cycles called for more elaborate consideration. But the simple phenomenon of cardiac inhibition, importantly represented in the superficially lying dorsal nucleus, readily accessible to temperature regulation from without, showed consistent decrease with lowered temperatures as indicated by cardiac acceleration. Cardiac inhibition from increased cerebral pressure, medullary as-

phyxia and from local cyanide poisoning match the effects of increasing temperature and allow the acid interpretation.

#### THE RELATIVE SENSITIVITY OF THE CENTRAL AND PERIPHERAL CHEMOSENSITIVE MECHANISMS

After Heymans had demonstrated the reflex regulatory capacity of the carotid body it was pertinent to question the need of and the relative importance of this newly discovered mechanism and Cromer & Ivy (68) were among the first to do so. They found that dogs worked just as well on the treadmill after the aseptic removal of the carotid bodies as they did before. And in this current year Dautrebande (52, 53) finds that dogs deprived of the carotid and aortic chemoceptors acclimate and adjust pulmonary ventilation to increased altitude as does the intact dog. Yet it has seemed unwise to conclude that the chemoceptors normally make no significant contribution toward the control of breathing. A decerebrated pigeon may give many outward signs of intelligence, as I learned on keeping such a bird for a year. After passing through its initial days of stupor it quickly gained the reputation of a genius, even with one who had raised these birds for profit. Little did its lay admirers know that to the very end it lacked the foresight to leave a plate of glass resting on a pot of boiling water. In respiration too, the whole of the regulatory mechanism must surely function better than any of its parts, but it is no simple matter to learn the relative importance of one part by removing either it or any other part.

Many have tested the response of denervated animals in acute experiments under anaesthesia to excess carbon dioxide and lack of oxygen. Barring such details as differences in rate and depth of breathing, carbon dioxide gives augmentation comparing with that in the intact animal. On the basis of such experiments it seems to be the consensus of opinion, we wonder whether right or wrong, that the respiratory center is as sensitive or even more sensitive to carbon dioxide than are the chemoceptors. On this point of relative sensitivity may hinge the relative importance of reflex and central response to carbon dioxide in the tonic control of breathing. For the present we do not know whether these two responses are additive and if so, how. In the light of Dautrebande's experiments, the relative importance of reflex and central response to oxygen

lack in the unanaesthetized animal becomes a similar problem of relative sensitivity and addition of effects.

Quantitative studies of the sensitivity of the chemoceptors are few, yet observations from various sources indicate a delicate response to oxygen and carbon dioxide changes. Significant among these findings is the persistence of action potentials in the afferent nerve of the carotid body during low pressures of carbon dioxide in the circulating blood. Local ischemia greatly increases this discharge [Bogue & Stella (69); Samaan & Stella (70)].

Another and very exacting test employs artificial perfusion of the carotid body and changes in ventilation and vascular constriction as signs of changing activity of the chemoceptors. The tiny carotid bodies must be isolated from the circulation with meticulous care if they are to retain their normal capacity to sense minute chemical changes in the nutrient blood. And the center must remain in trim if it is to register the incoming nerve signals. Bernthal (31) who has been investigating the principles of chemical stimulation at the chemoceptors, with these methods, has collected incidental data on their degree of excitability. He finds indications of high sensitivity of response to both oxygen and carbon dioxide fluctuations in the circulating blood. His indices are volume flow of blood and pulmonary ventilation. Blood equilibrated with oxygen at 18 per cent produces vasodilatation and decreased breathing or no effect at all. Blood equilibrated at 15 per cent produces consistent vasoconstriction and increased ventilation. One change of 10 mm. pressure on shifting from 12.8 per cent to 11.4 per cent oxygen definitely increased vasoconstriction and breathing. The sensitivity to carbon dioxide varied greatly and was markedly reduced by excess of oxygen (50 per cent) which was routinely used. The smallest variation of carbon dioxide pressure employed was 15 mm. and inasmuch as these particular tests were made with excess of oxygen and on relatively insensitive preparations, Bernthal believes that a much higher sensitivity could be demonstrated under more favorable conditions. He concludes with Samaan & Stella that normal carbon dioxide pressures of the blood are a source of reflex tonic control of circulation as well as ventilation and suggests that the oxygen pressures usually prevailing in the blood provide another source of reflex tonic control.

Appearing in the same number of the *American Journal of Physiology* with Bernthal's paper is another study of chemosen-



sitivity of the carotid body by Comroe & Schmidt (49) who obtain significantly different results. Using respiration as an index, they find a much lower order of response to oxygen lack and carbon dioxide excess. Using circulation, they were unable to demonstrate any stimulation with either oxygen lack or carbon dioxide excess. Summarizing the part played by reflexes, Schmidt (65) makes the following statements. "Under normal circumstances the reflexes are not concerned at all; modifications of respiration appropriate to compensate for ordinary changes in bodily action are accomplished by the direct effect of chemical substances (mainly  $\text{CO}_2$ ) upon the center without involving the relatively insensitive reflex receptor." "The chemical receptors are only stimulated by the most extreme degrees of  $\text{O}_2$  lack or  $\text{CO}_2$  excess." One cannot help but question the vitality of their test objects and suggest that their most significant postulates must seek other factual support if they are to survive.

There is a simple experiment which seems to throw some light on this problem of relative values [Gesell & Lapides (71)]. It consists of noting the duration of apnea after excessive artificial ventilation sufficient to produce hypocapnia, first with Hering's nerves and the vagi blocked and next with Hering's nerves conducting. Hering-nerve block under ordinary circumstances is capable of producing two effects. It can increase breathing by removing the inhibitory impulses coming from a stretched sinus. It can decrease breathing by removing the excitatory impulses coming from a stimulated carotid body as demonstrated by simple cooling of the carotid body (33). Despite the removal of inhibition coming from the carotid sinus, apnea is considerably longer during Hering-nerve block following hyperventilation. The onset of breathing occurs at a greater relative strength of anoxic stimulation than normally. Several questions arise immediately. Is the carotid body responding to oxygen lack earlier and more strenuously than the center? Does the earlier onset of breathing after removal of the block of Hering's nerves indicate that the carotid body is acting as pace setter in the tonic chemical control of carbon dioxide origin? Does the carotid body saturate more quickly with carbon dioxide as Heymans & Bouckaert (72) suggest and therefore come into action sooner than the center? Is the onset of breathing an additive resultant of the reflex and central chemical drives?

We may also ask a more general question. Is there not a possibility of an alternation of or a changing relative predominance of chemical control between the center and chemoreceptors with changing respiratory conditions? There is one observation which answers yes to this question. If Hering's nerve is blocked at the height of hypercapnic hyperpnea (vagi blocked) when the carotid body must be discharging most vigorously, there is no reduction in breathing. This may be explained in two ways. 1. The center may respond more vigorously to high carbon dioxide pressures than do the chemoreceptors. Recall that the reverse often occurs under relatively hypocapnic states. 2. Hypercapnia is unable to produce a reflex stimulation due to the central blocking effects of carbon dioxide on reflex stimulation (73). Central stimulation of the vagus nerve for example may produce no effects upon breathing during intense hypercapnia. Accordingly carbon dioxide would stimulate at the center and chemoreceptor and at the same time block the reflex impulses which it initiates at the chemoreceptors.

We have already alluded to the excessive elimination of carbon dioxide consequent on anoxic hyperpnea and the general alkaline trend of tissues favoring oxidations. Including the brain among the hypocapnic tissues we envisage a most paradoxical couple—anoxic hyperpnea and an hypocapnic center. The demonstration of such a situation would throw most interesting light on the relative values of central and reflex stimulation. To test this possibility, Hering's nerve was blocked during eupnea and the effects compared with those of block during anoxic hyperpnea [Gesell & Lapidus (71)]. The reduction of breathing to a distinctly lower absolute value when block was applied during hyperpnea indicates excessive washing out of carbon dioxide from the respiratory center. Barring unforeseen variables one may conclude that direct central stimulation during anoxia (and anaesthesia) is not a contributing factor toward anoxic hyperpnea. More pertinent still, we suggest that increased breathing occurs despite or in conjunction with central hypocapnia. The reflex hyperpnea turns the center alkaline, thereby protecting it as well as the rest of the body against the depressing effects of anoxia.

It is therefore concluded that there is an ever-changing relative intensity of central and peripheral drive. The greater the anoxemic hyperpnea, during anaesthesia at least, the smaller should be the

direct central drive and the greater the hypercapnia the smaller might be the peripheral drive. In the unanaesthetised dog, however, according to the findings of Dautrebande, the quantitative relationships between central and peripheral anoxic control might be drastically altered. Yet there are indications here too of a central hypocapnia, for breathing is frequently rapid and shallow.

#### MODIFICATION OF RESPIRATORY REFLEXES THROUGH CHEMICAL CHANGES AT THE CENTER

In that very connection, changes in the carbon dioxide content of the center are of the greatest importance in the modification of respiratory reflexes and therefore in pulmonary ventilation. For example, stimulation of the saphenous nerve produces a much higher rate of breathing during carbonate apnea [Gesell & Moyer (73)]. Conversely, stimulation of the central ends of the saphenous and vagus nerves during hypercapnic hyperpnea produces less effect than similar stimulation during eupnea. Hypocapnic apnea therefore, is not a condition of decreased central excitability as was commonly believed. In fact it may be an increased response to inhibitory impulses arising in the lungs—for the apnea produced by stretching of the lungs is markedly increased by the injection of carbonate. Though the chemical reflex drive would be similarly augmented, the impulses are shut off at their origin. The duration of apnea is accordingly a resultant of a decreased central drive of hypocapnic origin plus an augmentation of an inhibitory reflex. This conception explains the highly accelerated and shallow breathing of anoxia and the deep slow breathing of hypercapnia (73). In anoxia the hypocapnic condition of the center increases the normal accelerating influence of the vagi to which we have just alluded. During hypercapnia the vagal reflex is abolished or impaired, which allows a deep and slow type of breathing. We must then conclude that the chemical conditions of the respiratory mechanism controls ventilation in at least three ways: first by a central chemical drive which implies an inherent automaticity of the center, second by a reflex chemical drive arising in the carotid and aortic bodies which augments the central chemical drive and third by a central modification of respiratory reflexes. For an electrochemical mechanism providing a rhythmic firing of the center see Gesell (74, 16).

## OTHER CHEMICAL MECHANISMS OF CONTROL

Pi Süner (74, 75) adds another reflex chemical drive, initiated in chemoceptors in the tissues of the lungs, a most strategical position for the sampling of gaseous exchange. Though not confirmed and though refuted by Heymans & Heymans (5), Keller & Loeser (76), Partridge (77), and Dirken & Dishoeck (78), it has the indirect support of the histological studies of Larsell (79) who finds specialized sensory endings conceivably of chemoceptive function. It has also the indirect support of hyperpnea produced by pulmonary emboli [Binger (80)] and the rapid breathing of pulmonary oedema [Churchill (81)].

Other sources of chemical control may be discovered. Chemo-sensitive nerve endings within the brain itself is a possibility not to be overlooked.

PHYSICAL REFLEX MECHANISMS JOINTLY CONTROLLING  
VENTILATION AND CIRCULATION

Assisting the chemical mechanisms of control are two physical systems of adjustment—one operating on the proprioceptive impulses generated in the stretch of the carotid sinus and aortic arch and the other operating on impulses arising in the lungs and respiratory muscles. Though traveling along specialized highways, one set of impulses coming from circulatory structures, the others from the lungs and from the muscles controlling the size of the lungs, each group exerts combined effects upon circulation and ventilation. The possible arrangement of the central connection of these afferents has already been considered.

Distension of the carotid sinuses lessens vascular constriction, blood pressure, heart rate and pulmonary ventilation in relations now fairly well established. Under suitable experimental conditions there is a delicate and quantitative response of all of these functions to changes in absolute pressures in the sinus. The activity of each reflex is represented by a sigmoid curve—inhibition beginning at approximately 20 to 50 mm. Hg pressure, with maximum at approximately 200 to 250 mm. Hg, and increasing most rapidly at about 120 to 125 mm. Hg [Koch (82); Lim & Hsu (83); Schneyer (84); Winder (85, 86)]. The mechanism is accordingly most sensitive in the range of normal pressure changes. When the aortic

nerves are intact the results are modified. A rise of endosinual pressure lowers systemic pressure and sets up opposing aortic reflexes, sufficiently strong at times to convert an initial cardiac inhibition into acceleration and diminished breathing into breathing greater than normal. Reversals such as these indicate a predominance of aortic control in heart rate and breathing. Such complete reversal of the vasomotor reflex is impossible and accordingly the demonstration of the vasomotor relations to pressures in the sinus requires least rigid control of secondary variables.

Schmidt's statement (28) that "the respiratory response to alterations in endosinual pressure bore no constant relation to that of the circulatory" was based on experiments in which the aortic nerves and carotid body circulation were intact and arterial pressures and pulmonary ventilation uncontrolled. The apparent lack of resemblance of circulatory and respiratory reflexes which he noted cannot therefore be accepted as evidence for a variety of vascular proprioceptive end organs for the initiation of circulatory and respiratory reflexes respectively [Winder (85)].

There is no stronger evidence for a unity of action and purpose of the circulatory and respiratory systems than the similarity of the family of curves established by graded stimulation of the proprioceptive endings of the carotid sinus. It agrees with the similarity of respiratory and circulatory response to chemoreceptor stimulation. It accords with MacDowall's (87) experimental observations that the responses of the respiratory and circulatory systems to central asphyxia are really similarly directed. It ties in too with the interaction of the purely physical adjustments and the chemical systems of control.

If one distends the carotid sinus, ventilation decreases, carbon dioxide accumulates and the hydrogen-ion concentration rises to a new level upon which the entire respiratory system now operates. Inflate the lungs—ventilation decreases too and produces similar chemical conditions upon which the respiratory system operates. Deflation produces the opposite effects [Gesell & Lapides (71)]. In other words sinus and lung stretch decrease the effectiveness of carbon dioxide and acidity as respiratory stimulants. Thus purely physical mechanisms of adjustment are capable of playing a part in the control of the chemical equilibrium of the body.

## SIMULTANEOUS CONTROL OF RATE AND DEPTH OF BREATHING

Total movement of air is a product of rate and depth of breathing. Of necessity depth of breathing is primarily dependent upon the intensity of the central and peripheral chemical drives which determine the intensity of the central discharge, but it is also dependent upon the interruption or lack of interruption of this discharge by vagal and other closely related proprioceptive reflexes. In this more indirect way the vagi assume importance in the control of depth of breathing. Conversely the accelerating action of vagal interruption is assisted by an increased chemical drive which speeds the velocity with which respiratory contractions occur. The type of breathing which prevails at any time may then be looked upon as a resultant of highly variable chemical and physical drives. This resultant, however, is not always the expected. For example, the accelerating effects of chemical stimulation of the carotid body when added to the slowing effects of stretching of the lungs does not give an intermediate rate of breathing, but rather an acceleration considerably greater than that produced by chemical stimulation alone [Gesell, Steffensen & Brookhart (88)]. The unexpected rhythm is probably due to the striking acceleration of the inspiratory act common to lung inflation. This most important phenomenon assists in the attainment of deep and rapid breathing essential to greatly augmented tidal air.

The complexity of the rate-depth adjustment of breathing is further suggested by the enormous acceleration of breathing produced by stimulation of the saphenous nerve when the vagi are blocked and at the same time the medulla is cooled [Nicholson & Brezin (67)]. The absolute need of vagal impulses for very rapid breathing is thereby denied.

## TYPES OF PULMONARY PROPRIOCEPTIVE ENDINGS

The fact that lung inflation increases vagal potentials and that deflation decreases them, unless carried to extremes, speak for only one group of proprioceptor endings [Adrian (89)]. This view is supported by Partridge (77), Hillenbrand & Boyd (90), and Gesell, Steffensen & Brookhart (88), but contradicted by Hammouda & Wilson (91, 92). Gesell *et al.* were unable to duplicate the finding of Hammouda & Wilson with the most carefully graded

interruption of vagal conduction. Cold, pressure and progressive cutting had the same effects upon the reflex inhibition of breathing produced by pulmonary inflation. The graded increase of action potentials accompanying graded inflation of the lungs noted by Adrian (89) explains the accompanying graded deacceleration of respiratory rhythm described by Gesell & Moyer (73). The demonstration of the dependence of diaphragmatic tone on lung volume [Hess (93)] puts a very different emphasis on vagal function.

#### ABSENCE OF AN ADEQUATE ADJUSTMENT OF OXYGEN LACK

One of nature's gross errors, assuming that mistakes occur in physico-chemical systems, was her concentration on the response to oxidative carbon dioxide in combating oxygen shortage, combined with her neglect of the response to oxygen lack. This error appears attributable to the depressing effects of oxygen lack which stands in the way of a more rounded control of respiration. Possessed of high metabolism, brain tissue liberates large quantities of lactic acid when oxygen is scarce. This should, and we believe does, automatically provide acid stimulation to the respiratory and circulatory centers, and if brain works like brawn, it would provide the primary source of power to function as well, but evolution has failed in this most important aspect.

Nevertheless it is an ill wind that blows nobody good and Yandell Henderson (94) seems to be among those who have profited by the shortcomings of nature for he has investigated methods to counteract the harmful effects of oxygen lack. With a simple and physiological procedure of administration of oxygen and carbon dioxide mixtures he has combated with gratifying success the insidious and dangerous effects of asphyxia. Were it not for his explanations of the underlying principles his most interesting book relating his "Adventures in Respiration" (94) could be endorsed with few reservations. To me, his practical findings in resuscitation seem to fit the views outlined in the present article.

Henderson avoids the implications of acidosis as assiduously as we pursue them, yet the train of reactions associated with the appearance and disappearance of lactic acid with oxygen deprivation and replenishment, seem to offer the very basis for his enviable achievement of saving thousands of human lives. The fact that anoxemia and carbon monoxide poisoning are associated with



a decrease of blood acidity, and no increase of blood lactic acid when respiratory adjustments are normal, is no proof that the acidotic phenomena of oxygen lack are not involved. The interpretation by Henderson overlooks the postulate that an acidotic condition of minute collections of chemosensitive structures, be they chemoceptors or neurons of the respiratory center, is sufficient to control ventilation against oxygen lack without the accumulation of lactic acid in the blood and with a decrease of free hydrogen ions. It is unreasonable to expect the body as a whole to suffer from oxygen lack when special structures are functioning to maintain oxidations at par.

It must be remembered that the Henderson method is not applied to healthy normal individuals, responding perfectly to every increase of respiratory stress but to cyanotic and pallid individuals in whom the respiratory and circulatory mechanisms have failed from the depressing effects of oxygen lack. And where there is oxygen lack and depression there is lactic acid production, a phenomenon which cannot be disputed. The studies of McGinty & Gesell (41), on cerebral anemia and carbon monoxide poisoning, and of McGinty (95) on the effects of cyanidemia, anoxemia, and hemorrhage on the lactic acid content of the brain attest to that. There lies the danger of administration of oxygen alone. Oxygen given to an asphyxiated individual can suddenly remove large quantities of lactic acid by the non-oxidative route and thus liberate an equivalent amount of base which must be satisfied with carbon dioxide. The  $[H_2CO_3]/[NaHCO_3]$  ratio changes towards decreased acidity and removes the respiratory and circulatory stimulation. The beneficial effects of carbon dioxide on circulation and respiration described by Henderson (94) cannot take hold until it is too late. While the stimulus rebuilds, the animal deteriorates, and dies of oxygen lack. Add carbon dioxide to the oxygen mixture, as Henderson does, and the good effects of oxygen are retained and the bad avoided (15).

As we see it there are two immediate threats to a smooth supply of usable energy—oxygen lack and acid excess. Of the two the first is far more dangerous, for without oxygen, oxidations cannot continue and the machinery for the renewal of oxygen and removal of carbon dioxide is jammed. The harmful effects of

reduced oxidations from carbon dioxide excess, however, are far outweighed by the replenishment of oxygen produced by augmented ventilation. This is shown by the elevation of the oxygen content of the blood and the improvement of the subjective condition at high altitudes by the administration of carbon dioxide [Schneider, Truesdell & Clark (96); Dill (8)].

#### MODIFICATION OF EXCITABILITY TO THE NORMAL RESPIRATORY STIMULUS

It is maintained by some, that oxygen lack increases the excitability of the respiratory center to its normal stimulus, carbon dioxide, and thereby controls ventilation. One obstacle to such a mechanism is the depressing action of anoxemia which so often prevails. The general ease with which excitability is affected by chemical influences of various sorts emphasizes the possibility of this control. Be that as it may, direct experiments do not support the postulate, at least not in the anaesthetized dog. Anoxemia which stimulates the carotid body, increases pulmonary ventilation and thereby establishes an hypocapnic state of the respiratory center, almost apneic and incapable of maintaining adequate ventilation [Gesell & Lapidès (71)].

These findings are not recapitulated to deny increased excitability of the respiratory mechanism from oxygen lack. That idea is acceptable, but on a very definite basis [Gesell (15)]. For example—inject an animal with hydrochloric acid and breathing increases, more and more as the injections are repeated. The momentary increases after each injection are attributable to the sudden liberation of carbon dioxide in the circulating blood supplying the chemosensitive respiratory mechanisms. But after each excess of carbon dioxide is eliminated why does the dog continue to breathe with increased vigor? The carbon dioxide content of the blood tells why. At first it is greatly decreased with each injection, but as the breathing becomes more and more vigorous the reduction becomes less and less marked. This means that hydrochloric acid is reacting with tissue base. Assuming a uniform production of metabolic carbon dioxide there must of necessity be an increase in the  $[H_2CO_3]/[NaHCO_3]$  ratio in the elements of respiratory control. The increasing acidity whips up ventila-

tion. Put in terms of excitability—fixed acid reduces the amount of buffering sodium bicarbonate and thereby increases the excitability of the denominator of the buffer mixture to carbonic acid.

The argument is the same for fixed acid provided by the metabolic route. Liberate free lactic acid in the carotid body by oxygen lack and you decrease the denominator of the buffering mechanism of the chemoceptor and increase its excitability to free acid. Consequently the greater the oxygen privation at the chemoceptor, the lower becomes its base, and the more acid it turns to its own carbon dioxide. Thus it turns the tables on the blood and tissues by increasing ventilation. Within the limits of physiological adjustment the blood and tissues turn proportionately less acid as the chemoceptors turn more acid. Only the sentinels of distress must weather the storms of privation.

#### DIRECT ACTION OF OXYGEN LACK

It has also been maintained that oxygen lack acts directly as opposed to the indirect effects of acidity in the control of breathing. This theory is revived by Comroe & Schmidt (49), not for the respiratory center, since they still retain carbon dioxide and acidity as the normal stimulus for that structure, but for the carotid body and other chemoceptors so easily demonstrated to respond to oxygen lack. To quote, "Since the anoxic bloods caused a consistent and marked reflex hyperpnea, although the blood was more alkaline and the hypercapnic bloods were less effective in arousing reflexes, although the blood was more acid, it is evident that the changes in hydrion concentration *per se* have no significant effects upon the carotid body receptors over the physiological range studied in these experiments."

Why Comroe & Schmidt ignore the changing relationship of intracellular and blood acidity established from so many points of view, why they overlook the significance of the differences between intra- and extra-cellular hydrogen-ion concentration of the protozoan, established by Jacobs in their own institution, when they accept the acid mechanism of stimulation for the respiratory center, is difficult to understand. The point which Comroe & Schmidt stress in particular is that ventilation increases, more to oxygen lack than to carbon dioxide excess. On that basis they make the following statement:

"We suggest that the carotid body receptors are essentially structures in which an interference with oxidations gives rise to afferent nerve impulses stimulant to the respiratory center. This is based on the intensity of the reflex hyperpnea elicited by anoxemia and on the similar effects of cyanide and sulphide, which are characteristic inhibitors of tissue oxidations. Nicotine, another stimulant to the receptors, inhibits oxidations in some living systems; lobeline apparently has not been tested in regard to biological oxidations. The effects of high tensions of carbon dioxide may also be explained on this basis as the result of interference with oxidations in the receptors because of the failure of removal of  $\text{CO}_2$  produced by them."

It is not the finding of greater response to a given change in oxygen partial pressure that disturbs us, for Bernthal also finds this relationship. However, it must be remembered that he was able to demonstrate a much higher sensitivity to both oxygen lack and carbon dioxide excess than Comroe & Schmidt. It is the conclusions arrived at without regard to chemical and metabolic mechanisms, to the characteristics of the carbon dioxide and oxygen dissociation curves or to the forms in which carbon dioxide is carried by the blood.

Think of the inconsistencies. Compare for example the effects of intravenous injection of one gram of sodium cyanide and one gram of hydrochloric acid. Enough in the case of cyanide to produce sudden asphyxial death, and if injected slowly, a prolonged reduction of oxidations and a tremendous increase in ventilation. One gram of hydrochloric acid increases ventilation only slightly and its effects on oxidation are also very small. That to be sure is not contrary to the view of Comroe & Schmidt. But quantitative comparison, made by Pelecovich, suggested the significance of tissue acidity rather than of oxidation *per se*. If then, the comparison of sodium cyanide and hydrochloric acid is made in terms of acid, we find that the effects of cyanide should be disproportionately greater than those of hydrochloric acid. Capable of liberating large amounts of lactic acid, it has the potential strength of many times its weight in acid. As exemplified by one computation from our experiments on uniformly ventilated dogs in which sodium cyanide reduced the carbon dioxide capacity of the body one hundred times as much as did an equivalent amount of hydrochloric acid (97, 98). If then a comparison of carbon dioxide and

oxygen effects resolves itself to carbon dioxide and lactic acid, acidity as a peripheral reflex mechanism of control cannot be dismissed. A quantitative comparison, however, requires most critical data as witnessed by two most important factors: (1) Carbon dioxide exerts a dual action on acidity, an alkaline effect by an indirect increase of the denominator and an acid effect by a direct increase of the numerator of the  $[H_2CO_3]/[NaHCO_3]$  ratio; (2) Lactic acid on the other hand produces two acid effects—it increases the numerator by releasing free carbon dioxide and decreases the denominator of the  $[H_2CO_3]/[NaHCO_3]$  ratio by reacting with sodium bicarbonate. That is tremendously in its favor as an acid stimulus when compared with carbon dioxide, particularly when we remember that oxygen lack gives rise to a disproportionately large formation of fixed acid.

#### SPECIFICITY OF ACTION OF CARBON DIOXIDE

Specificity of action of carbon dioxide as opposed to its acid effects was another of the numerous theories of respiratory control. It had the support of the observations of Laquer & Verzář, Hooker, Wilson & Connett, Scott, Dale, Evans, Collip and others. But the nature of its effects was never specified. Highly speculative, as it seemed, acidity was the only tangible clue to a mode of action and when fairly tested in every conceivable way was found to meet the requirements of the control of breathing by cellular acidity. The arguments against specificity were presented in detail in our older review and may be consulted by those who are interested in this particular phase.

Nielsen (99), comparing ventilation with the hydrogen-ion concentration of the blood during hypercapnia and ammonium chloride acidosis, finds greater changes in ventilation during hypercapnia. He arrives at these conclusions: "Wasserstoffionen sind nicht der chemische Reiz der Atmung." "Der adäquate Chemische Reiz der Atmung muss deshalb in einer spezifischen Wirkung der gelösten Kohlensäure in Atmung gesucht werden." That breathing and carbon dioxide vary inversely with one another in anoxemic hyperpnea is contrary to the specific action of carbon dioxide yet compatible if we employ the accessory theory that oxygen lack increases excitability to the normal stimulus

carbon dioxide. This is done by Nielsen without, however, specifying the manner in which excitability is stepped up. We have no difficulty in accepting the general idea of controlled excitability and have offered one possible scheme—not increased excitability of some undefinable mechanism, but of the denominator of the  $[H_2CO_3]/[NaHCO_3]$  ratio. This applies readily to another of Nielsen's observations that ammonium chloride acidosis increases the excitability to the normal respiratory stimulus carbon dioxide. Obviously both applications of our concept must be unacceptable to Nielsen unless he wishes to join with others and adopt the almost universally acceptable principle of respiratory control—the stimulating action of acidity.

In this review we have tried to present, in the light of current work, what appears to be the most promising theory of respiratory control. We envisage a deep underlying principle, as old as life itself, employing the use of carbon dioxide excess and oxygen lack. Inextricably inseparable and apparently working on a common basis of cellular acidity, their interaction allows a logical interpretation. It is therefore with some concern that we note the revival of theories which for some years seemed forgotten. The insistence of a supersensitive response of the respiratory center to the hydrogen-ion concentration of the blood [Haldane & Priestly (100)]; the conviction that acidity is not the stimulus to respiration [Nielsen (99)]; the combination of these two views—that acidity is the stimulus at the center, but not at the chemoreceptors [Comroe & Schmidt (49)]; that oxygen lack, independent of acidity effects, is the stimulus at the chemoreceptors [Comroe & Schmidt (49)]; the control of excitability of the respiratory center by oxygen lack to the normal respiratory stimulant—carbon dioxide [Henderson (94) and Nielsen (99)]; the specificity of carbon dioxide as opposed to its effects on acidity [Nielsen (99)]; the uncertainty of decision between specificity of carbon dioxide and acidity [Carlson and Johnson (101)]—all taken together, inspire a new version of Rip van Winkle, who having worked instead of slept while sleeping, woke twenty years later and found the world unchanged. A commentary on the elusiveness of the mechanisms of respiratory control.

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DEPARTMENT OF PHYSIOLOGY  
UNIVERSITY OF MICHIGAN  
ANN ARBOR, MICHIGAN

## MUSCLE\*

BY EMIL BOZLER

*Department of Physiology, Ohio State University  
Columbus, Ohio*

The present review is a report on the progress in the more general aspects of muscle physiology during the two years preceding September 1938. The subject has been divided into four main sections (a) physical properties, (b) excitability, (c) myoneural junction, (d) miscellaneous. Following a suggestion by the editors, the chemistry and metabolism of muscle will not be discussed in this review, because it is planned to continue treating this phase of the subject in the *Annual Review of Biochemistry* in alternate years.

### PHYSICAL PROPERTIES

The study of the physical properties of muscle is as yet the only method of approaching the problem of the mechanism of muscular contraction. The interest in this field has centered mainly on the analogies between muscle and some fibrous materials. The study of the birefringence and of the x-ray diagrams of muscle have shown that the contractile structures contain long molecules oriented parallel to the axis of the fibers. According to Meyer's well-known theory, contraction is due to the folding of these molecules, produced by electrostatic forces between active side groups. Valuable discussions of this theory which, in its essential features, is accepted by most recent authors have been given by Fischer (1) and Schmidt (2) and a similar but more specific theory, not applicable to smooth muscle, has been presented by Bernal (3); however, the assumption involved in Meyer's theory that muscle is structurally closely related to fibrous substances has been criticized because this comparison disagrees with some essential properties of smooth muscle (4, 5).

*Submicroscopic structure.*—The x-ray diagrams of frog skeletal muscle, first obtained by Boehm & Schottky (6), are rather poor, but they resemble those of keratin. Several other muscles, including a smooth muscle, gave the same diagrams as frog muscle (7), which suggests that the patterns obtained are those of the contractile elements, not of some supporting structure. The diagrams

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become slightly more distinct on stretching (7), evidently the result of a greater degree of orientation.

The birefringence of muscle has been studied by several workers. Bozler & Cottrell (8) have described a simple apparatus for recording fast changes of birefringence. The measurement of the average birefringence of a whole cross-section of a muscle with the same apparatus has the advantage over previous methods that it excludes errors due to the uncontrollable changes of shape during stretch and contraction.

The interpretation of optic phenomena is undoubtedly much simpler in smooth muscle than in striated muscle. Fischer (9) measured the change of birefringence of a smooth muscle from a marine worm during isometric contraction. The birefringence decreased at short lengths, but the variation was very small or absent at intermediate lengths and was frequently even positive when the muscle was fully extended. However, Bozler & Cottrell (8), using the method mentioned above, reported that the birefringence does not change during the isometric contraction, neither in a retractor muscle of a snail nor in the muscle used by Fischer.

Bozler & Cottrell (8) confirmed Muralt's observation that the birefringence of the frog sartorius decreases during the isometric contraction. The variation, however, was appreciable only at the small initial tensions used by Muralt and was almost imperceptible at greater lengths. Because, even in an isometric contraction, a shortening of some parts of the fibrils and an extension of others probably always occurs, the optic changes are perhaps caused by the changes of length and not by the activity of the muscle. Studies on single muscle fibers like those of Buchthal & Knappeis (10) may help to clear up the uncertainty of the interpretation of the optic changes in striated muscle. These authors observed the decrease of birefringence during the isometric contraction in single muscle fibers.

The observations of Buchthal & Knappeis (10) suggest that the optic properties of skeletal muscle are influenced by chemical factors. Fatigue produced a considerable decrease of birefringence which could be prevented by iodoacetic acid. An even greater and reversible decrease was produced by adding lactate to the Ringer's solution surrounding the muscle fibers. Liang (11), on the other hand, showed that the decrease of birefringence in various kinds of rigors is due to the tension rather than due to the lactic acid produced.

Buchthal, Knappeis & Lindhard (12) have measured the length of the I- and Q-discs from microphotographs of living muscle fibers during rest and during the isometric and isotonic contractions. In agreement with the generally accepted view it was found that only the anisotropic Q-discs contract actively whereas the I-discs always become longer during contraction. Many of the older, contradictory, observations were made on preserved and stained tissues and seem, therefore, unreliable. On the other hand the measurements on stained preparations are more accurate than those on living material. According to Schmidt (2) the great thickness of the striated muscle fibers and the presence of diffraction bands in the microscopic image introduce into the measurements on living muscle unpredictable errors which are largely avoided in studies on histological preparations. The question whether the I-bands are contractile, therefore, is still open. Schmidt also considered the possibility that the border between the Q- and I-discs shifts during contraction. The assumption that the I-discs participate actively in contraction seems reasonable because they are birefringent, although much less so than the Q-discs (13).

Stretching increases the birefringence of smooth muscle. A quantitative study of this phenomenon in a retractor muscle of a snail (8) has shown that the increase is proportional to the square root of the length. This means that the increase of birefringence just compensates for the decrease of thickness so that the phase difference of ordinary and extraordinary rays remains constant when the length of the muscle changes. Because extension of the muscle does not produce any permanent tension the change of birefringence must be due to a plastic deformation involving a change of position rather than a change of the shape of molecules. The effect can be explained quantitatively by assuming that the oriented molecules are lying in surfaces and that previously un-oriented molecules become oriented on stretching. According to earlier observers the variation of birefringence during the isotonic contraction has approximately the same magnitude as the changes during passive extension. It seems possible, therefore, that this optic effect also is not directly related to the active phenomena of contraction but rather to the changes of the length of the contractile structures.

Fischer (14) determined the relative importance of micellar

and form birefringence by the imbibition method using muscles fixed in formaldehyde at different lengths. At increasing lengths the micellar birefringence increases relatively little as compared with the form birefringence. The author concludes that the increase of birefringence on stretching is caused by an elongation of ellipsoidal micellae, and that the micellae are nearly perfectly oriented parallel to the muscle fibers already when these are only moderately stretched.

*Myosin.*—Myosin is important not only because it forms the largest protein fraction of muscle, but also because it is probably derived from the birefringent material of muscle. Deuticke and others observed that this protein was extracted incompletely in rigor and other conditions, indicating a decrease of solubility and perhaps a partial coagulation. Bate Smith (15), however, found no change of the properties of myosin during rigor and explained the earlier observations by the assumption that, under certain conditions, myosin becomes less accessible to the extraction fluids. It is interesting in this connection that no myosin is found in muscle press juice although it is fairly soluble in this medium. This fact suggests the presence of a membrane surrounding the fibrils. Bate Smith assumes that this membrane is altered during rigor so as to make the inside less accessible to the extraction fluids.

*Mechanical properties.*—The thermoelastic properties of skeletal muscle are qualitatively like those of other substances with rubber-like elasticity. It is characteristic for these substances that heat is liberated during stretch in contrast to other elastic bodies. Furthermore it has been observed that the temperature coefficient of an elastic force (measuring the change of the elastic force with temperature at constant length) is negative for small, positive for intermediate elongations (7). Thermodynamic considerations show that this result is in agreement with previous experiments on the thermoelastic effect and on the temperature coefficient of linear expansion at constant load. Meyer & Picken have pointed out that these thermoelastic properties are the direct result of the orientation of the molecules during stretch. The heat liberated during stretch is comparable with the heat of crystallization. Ebbecke (16, 17, 18), and Remberg & Ebbecke (19) have made further contributions to the analogy between skeletal muscle and rubber.

It has been pointed out, however, (4, 5) that the elasticity of the resting skeletal muscle is certainly in part, perhaps entirely,

a property of the supporting structures and that there is no proof that the mechanical properties studied so far have anything to do with the contractile elements. This problem cannot be solved by the study of the mechanical properties alone; additional physiological data are necessary. Such data have been obtained from certain smooth muscles in which the properties of the contractile structures, because of their high viscosity, can be studied separately from those of extraneous elements. In these muscles rapid extension produces tension which decreases exponentially at constant length. The rate of this process is exactly equal to that of the relaxation of an isometric contraction showing that it is controlled directly by the contractile elements of the muscle. Extension of the relaxed muscle does not produce any permanent tension within a wide range of lengths.

Stretching experiments on skeletal muscle, similar to those performed on smooth muscles, show that the viscosity is very low in the former type of muscle. For this reason effects due to viscosity are obscured by the mechanical properties of extraneous structures. In contrast to some smooth muscles, viscosity is not a factor controlling the rate of relaxation in striated muscle (5).

*Transmission of light.*—Nicolai & Gruethling (20) observed that skeletal muscle becomes more transparent during the isotonic contraction in spite of an increase of thickness. The variation of transparency reaches its maximum sooner than the mechanical response. Periodic changes were found at frequencies of stimulation up to 54 per second. The change of light transmission is probably caused by the change in shape of the isotropic and anisotropic discs. Schaefer & Göpfert (21) found great differences in the behavior of different muscle preparations; sometimes an increase, sometimes a decrease, occurs and no reason for the variability has been discovered. It was also observed that the total light transmitted can increase while the intensity of the diffraction-spectra decreases.

Millikan (22) and Urban & Peugnet (23) who studied light transmission in monochromatic light were able to follow the reduction of hemoglobin and of the yellow enzyme during a short tetanus, and they showed that oxidative phenomena begin within less than one second after the beginning of a contraction.

*Energy changes.*—The term viscosity has been used by Hill to express the fact that the tension produced by a stimulated muscle



diminishes as the speed of shortening increases. Whereas Hill originally favored a purely physical explanation of this relation, Fenn assumed that the rate of shortening is controlled by the rate of energy mobilization. Because more power is needed at higher speeds, the rate of shortening must decrease with increasing load, provided the power output is a limiting factor. Fenn (24) showed that the experimental results, particularly the relation between load and speed of shortening, are not in agreement with the physical interpretation of Hill and his coworkers. Hursh (25) confirmed Fenn's results using dogfish muscle and two invertebrate smooth muscles. Hill (26) now agrees with Fenn's interpretation of viscosity, and his energy measurements with an improved myothermic method (27) confirm Fenn's conclusions.

Bozler (5) measured the rate of heat production during the isometric contraction of an invertebrate smooth muscle. The relaxation heat varied approximately as the 1.7th power of the tension. This relation is in agreement with the assumption that the relaxation heat is equal to the elastic energy of the muscle and that the elastic properties of the muscle remain unchanged during relaxation. The experiments confirm the previous conclusion that contraction brings about a decrease of the resting length of the muscle, not a change of its elastic properties as is generally assumed.

#### EXCITABILITY

*Electric stimulation of striated muscle.*—The strength-duration curve of skeletal muscle, as shown by Lucas, has a break, and the two parts making up the whole curve have suggested the presence of two excitable systems. Lapique considered the slow component, or  $\alpha$ -excitability as an artifact produced by polarization and present only if large fluid electrodes are used. However, Lippay (28) observed  $\alpha$ - and  $\gamma$ -excitability also if small stimulating electrodes were used. The  $\gamma$ -excitability was abolished by curare and nerve degeneration, confirming the conclusion of previous workers that it is a property of the nerve fibers inside the muscle. The  $\alpha$ -excitability, therefore, can be considered as a true representation of the characteristics of muscle. This conclusion is supported by theoretical considerations and observations of Blair (29) showing that the shape of the  $\alpha$ -strength-duration curve is essentially like that of nerve, and that its relation to latent addition, which measures the subsidence of the excitatory process, is likewise as in

nerve. The rate of subsidence was found to be two to three times faster than was expected from the strength-duration curve.

Brown and Sichel and others showed that single muscle fibers may give graded, local contractions, but Steiman (30) demonstrated that responses of this type occur only in injured preparations. Further observations on single muscle fibers (31) suggest that the excitatory phenomena of muscle can occur without contraction. A localized response may occur at some distance from the site of stimulation and this distant effect does not seem to result from a spread of the stimulus. Consequently physiological conduction in the absence of a mechanical response must be assumed.

*Smooth muscle.*—In view of the importance of smooth muscle for many physiological functions, it seems unfortunate that widely different views have been expressed even on the most fundamental characteristics of this type of muscle. Its study has been difficult because of the complicated anatomical distribution and the frequent occurrence of spontaneous activity.

Although Bacq & Monnier (32) and Eccles & Magladery (33) obtained weak contractions by stimulating the denervated nictitating membrane of the cat, it has been questioned whether this muscle as well as other kinds of smooth muscle can be stimulated electrically. Cannon & Rosenblueth (34) assumed that the responses to electric current are due to the stimulation of motor-nerve fibers or due to the injurious effects of the strong electric currents used. Bozler (35), however, showed that uterine muscle can be stimulated directly by electric shocks of suitable duration if the muscle is taken from an animal during estrus. Previous negative results with the same organ (36) are explained by the fact that animals in anestrus were used.

The difficulty of stimulating directly some types of smooth muscle may be due to the short length of the responding units. In this case only a small part of the stimulating current will pass through the muscle fibers, with the result that the threshold is high. An explanation for the difficulty of stimulating the nictitating membrane seems to be suggested by the anatomical study of Acheson (37) who demonstrated that the movements of this organ are caused by a muscle lying in the peribulbar fascia and extending only slightly into the membrane itself.

Because smooth muscle cells are very short (about 0.05 mm. on the average) it has been difficult to understand the excitatory

phenomena and the coordinated activity of visceral muscles. A simple explanation of many of the properties of this type of muscle is provided by the assumption of syncytial connections between the smooth muscle fibers (first postulated by Engelmann, 1870). By experiments on uterine strips and on the ureter Bozler (35) has shown that visceral muscles can conduct impulses like skeletal muscle fibers or cardiac muscle. That nervous elements are not involved in these responses has been concluded, among other facts, from the observation that the uterine muscle of the cat gives conducted responses although it has only inhibitory innervation, and from the finding that cocaine blocks conduction only in high concentrations (1:100). The assumption of the syncytial character of smooth muscle explains also the great simplicity of its responses to electric stimuli. Provided that conduction takes place the responses are like those of single striated muscle fibers; they are all or none, they start at the cathode and are followed by a refractory phase. The excitatory processes in these muscles are, therefore, qualitatively like those of nerve and striated muscle, and they differ from the latter only by their slower time characteristics.

Smooth muscles supplied by true motor nerves, in contrast with visceral muscles, consist of a great number of muscular units. This difference explains why the responses of the two types of smooth muscles are quite different from one another. The nictitating membrane of the cat is suitable for the study of the type of smooth muscle with motor innervation. This simple nerve-muscle preparation has been studied by a variety of methods. Only the most significant results can be mentioned.

(a) According to Eccles & Magladery (38, 39) every response is followed by an absolutely refractory phase, lasting for about 50 msec., during which the size of the initial complex of the action potential is greatly diminished. Rosenblueth *et al.* (40, 41, 42), however, questioned the validity of conclusions based only on the electric responses and they presented evidence showing that the mechanical response of smooth muscle is not always accompanied by an action potential. The authors found no refractory phase for the mechanical response longer than that of the nerve. Confirming Brown (43), the minimal interval for summation of the contraction was less than 2 msec. and summation reached a maximum at 40 msec. (44). Furthermore, a response to nerve stimulation was addi-

tive to a contraction produced by epinephrine, showing that the response to the drug did not set up a refractory state.

(b) According to Eccles & Magladery every muscular unit is innervated by several motor nerve fibers and a muscle impulse arises only by the summation of the effects of several nerve impulses. This conclusion was drawn from the interaction of the effects of successive submaximal nerve volleys. Rosenblueth & Rioch (45), on the other hand, assuming that one muscle fiber was supplied by not more than one motor nerve fiber, previously concluded from similar experimental evidence that the all or none relation was not valid for smooth muscle fibers.

(c) Eccles & Magladery (39) observed that single maximal nerve impulses often elicit several rhythmic potential waves following the initial complex; two successive volleys regularly produce a repetitive discharge. Each wave precedes an increase of the mechanical response and is followed by a refractory phase. The whole response, therefore, is a tetanus. Also epinephrine produces rhythmic activity at first, but in later stages of its action a contracture occurs which is not accompanied by action potentials. Only the late rhythmic waves of a response elicited by nerve stimulation, not the initial contraction, are assumed to be initiated by a chemical mediator.

It seems that many conclusions based on experiments on the nictitating membrane will have to be reconsidered in view of Acheson's recent anatomical findings (37) and the question of the interpretation of the complicated action potentials of this muscle in particular seems to be open for the same reason.

#### MYONEURAL JUNCTION

While it is generally admitted that motor nerve impulses cause the release of active substances at the nerve endings, the significance of this finding has been a subject of controversy. Whereas many investigators consider chemical agents as the exclusive transmitters of nerve impulses, there are others who assume that the electric changes are the essential factor for the transmission across junctional regions. To test the validity of these hypotheses, the effects of the chemical mediators have been compared with those of nerve stimulation. Furthermore, the facilitatory and inhibitory effects of nerve stimulation have been carefully studied and their explanation on the basis of the different theories of neu-

romuscular transmission has been discussed. Several reviews on this subject appeared recently (34, 41, 42, 46, 47, 48).

*Acetylcholine.*—Brown (49) completed the proof that acetylcholine produces in skeletal muscle tetanic contractions like those evoked by nerve stimulation. Studies of action potentials showed that acetylcholine applied by the method of close arterial injection elicits conducted impulses which originate, probably, at the myoneural junction. The frequency of this discharge is about 200 per second at the beginning and gradually fades out within about a second. Normal and denervated mammalian muscle and frog muscle gave essentially the same results (50).

Eserine changes the response of skeletal muscle to a nerve volley from a twitch to a brief tetanus (50, 51). According to Feng (52) this effect occurs in amphibian muscle only if the interval between the stimuli is several minutes. The reason for the difficulty of obtaining eserine potentiation in amphibian muscle is obscure. Also barium ions have an eserine-like action (53). Under their influence a single nerve volley produces a tetanus lasting for more than a second whereas direct stimulation of the muscle gives single twitches.

The simplest explanation of the eserine potentiation is provided by the protective action of eserine on acetylcholine, but Eccles (46) considers it more likely that eserine acts by raising the level of excitability of the muscle. The first of these explanations is well supported by observations of Bacq & Brown (51). The drug has the same effect on the response to nerve stimulation and to acetylcholine. Both types of response are followed by a depression of neuromuscular transmission, an effect which, under certain conditions, obscures the potentiation by eserine (51). All substances with nicotine action have this depressing action, stable substances without the presence of eserine. Substitutes of eserine act in proportion to their inhibitory effect on choline esterase.

*Facilitation.*—In a lightly curarized muscle, two or more impulses in rapid succession are necessary to produce a response. A single nerve volley, although it is not transmitted to the muscle, evidently sets up an excitatory process which can be measured by recording the height of the response to a second nerve volley. Bremer & Kleynjens (54) found that this excitatory process, facilitating transmission, lasts for about 60 msec. Because facilitation is not increased or prolonged by eserine [confirmed by Maaske

*et al.* (55)] it is improbable that the long after-effect of a motor nerve impulse is due to the presence of acetylcholine.

In avian muscle and even more in crustacean skeletal muscle (56) facilitation plays a role under normal conditions. Brown & Harvey (57) observed in the leg muscles of the fowl that the action potential of the muscle in response to the second of two nerve volleys is larger than the first if it follows within 150 msec. Because *trappe* and other possible explanations of the phenomenon have been ruled out, it must be assumed that a single volley activates only about 75 per cent of the muscle fibers.

*Wedensky inhibition.*—Previously, only refractoriness has been considered as the cause of Wedensky inhibition, but Cowan (58), Feng *et al.* (59, 60, 61, 62) and Rosenblueth & Morison (63) have offered an entirely new explanation of the phenomenon. They assume that the more or less complete failure of neuromuscular transmission at high frequencies is the result of accumulation of acetylcholine at the nerve endings. One of the chief arguments in favor of this conclusion is the observation that eserine and prostigmine strongly favor the appearance of this phenomenon. According to Feng (59) the block appears in normal amphibian muscle at a frequency of 200 stimuli per second, but under the influence of eserine it occurs at frequencies as low as 40 per second.

Feng's further observations on Wedensky inhibition (60, 61, 62) lend strong color to the view that specific processes occur at the myoneural junction. He showed that during Wedensky inhibition there is not only a block for the transmission of impulses from nerve to muscle but also for impulses conducted along the muscle fibers (elicited by stimulating the nerve-free pelvic end). This block is the direct result of a strong contracture at the region of the nerve endings detectable by a double myograph and by a double thermopile. Because acetylcholine in sufficiently large amounts is known to cause contracture and to abolish excitability, all the effects observed can be readily explained as the result of the release of acetylcholine at the myoneural junction.

*Curare.*—A failure of neuromuscular transmission at relatively low frequencies of stimulation similar to that in an eserinated preparation occurs in lightly curarized and fatigued muscles, but Rosenblueth & Morison (63) showed that the neuromuscular block can have two quite different causes which can be distinguished by the effect of acetylcholine. This drug increases the Wedensky in-

hibition of an eserinizd muscle, but facilitates transmission in a curarized or fatigued muscle. The block produced by curare is perhaps due to a decrease in sensitivity of the muscle fibers to acetylcholine, an explanation which is consistent with the observation that curarine restores the responses depressed by eserine (64, 65). The block produced by fatigue, on the other hand, seems to be caused by a diminished output of the transmitter. In summary, it may be said that the Wedensky inhibition under the influence of eserine probably is the result of a superabundance, whereas the apparently similar condition produced by curare and fatigue is due to a deficiency of acetylcholine (63).

*Post-tetanic facilitation.*—Following a short tetanus the condition of a muscle is changed for several seconds or even minutes as indicated by an increase in the height of the twitch. Guttman, Horton & Wilbur (66) who first drew attention to this fact, assumed that it was caused by the accumulation of the chemical transmitter at the nerve endings. As pointed out by Rosenblueth & Morison (63) and Feng *et al.* (67, 68) this explanation is improbable because of the long persistence of the effect. Brown & Euler (69) gave convincing evidence showing that the after-effect of a tetanus is caused by the release of potassium ions from the muscle fibers. That the site of action of the potentiation is not the myoneural junction is shown by the fact that it occurs also in denervated and curarized muscle and that there is no after-discharge. The potentiation is accompanied by a decrease of the action and demarcation potential; neuromuscular transmission may be restored in a partially curarized muscle, probably by a general increase in the level of excitability. All these effects can be imitated by close arterial injections of potassium chloride.

*Choline esterase.*—Because after-discharges normally do not occur on indirect stimulation of skeletal muscle, the transmitter must become ineffective before the end of the refractory period. The briefness of this phase has been one of the chief difficulties in accepting the theory of chemical transmission in skeletal muscle. Marnay & Nachmansohn (70) found that the concentration of choline esterase in muscle tissue was not high enough to remove the acetylcholine released by a nerve impulse during the refractory phase. Later (71), the authors showed that the nerve-free tissue contains considerably less enzyme than the rest of the muscle, indicating that the concentration of esterase is many thousand



times greater at the nerve endings than in the muscle tissue. Assuming that the rate of destruction of acetylcholine is proportional to its concentration it was calculated that the amount of acetylcholine released by a nerve volley can be removed within the refractory phase.

Clark *et al.* (72) made a careful study of the kinetics of the action of choline esterase. They found that the rate of hydrolysis of acetylcholine is proportional to the concentration of enzyme and nearly proportional to the concentration of substrate (0.83th power of the concentration). The rate of hydrolysis at physiological concentrations then comes out to be much lower than Marnay & Nachmansohn had assumed. At the highest concentrations of enzyme which one may reasonably assume to occur at the nerve endings it would take at least 0.1 sec. for half hydrolysis of even minute amounts of acetylcholine.

*Peripheral inhibition.*—For an understanding of the peripheral inhibition of smooth muscle it is interesting to know the changes in the properties of muscle produced by the action of inhibitory nerve fibers. Bozler (73) using perfused frog legs, studied quantitatively the inhibitory effect of vasodilator impulses on the musculature of blood vessels. The vascular responses were recorded by a flow-meter which was sensitive enough to measure the response to single volleys of constrictor impulses. Stimulation of vasodilator nerves produced complete suppression of the responses to vasoconstrictor impulses if the vasoconstrictors were stimulated at a low frequency, but the response to faradic stimulation was not appreciably diminished. Whereas usually a single volley of impulses was sufficient to elicit a response of the blood vessels, repetitive nerve stimulation was always necessary to obtain a contraction during a state of inhibition. Inhibition, therefore, may be considered as the reverse of neuromuscular facilitation.

Inhibitory nerve fibers can also modify pre-existing automatic activity. Since facilitation is not necessary in this case, the inhibitory nerves never abolish the responses to nerve stimulation, but merely lower the level of spontaneous activity on which the responses are superimposed. The antagonism between cardio-accelerator and cardio-inhibitory nerves and that between the sympathetic and parasympathetic nerve supply of the retractor penis of the cat as studied by Oppenheimer (74), are examples of the control of muscular activity under this condition.

Inhibition in crustacean skeletal muscle produced by specific inhibitory nerve fibers is essentially a depression of facilitation (75, 76, 77) as was found for smooth muscle under comparable conditions. During inhibition the muscle action potentials which accompany a tetanic contraction may drop out entirely in groups (78) demonstrating the depression of neuromuscular transmission, but under other conditions the potentials are only diminished in size or even remain unchanged. Serkoff (78) and Marmont & Wiersma (77) made the surprising observation that the contraction may be completely inhibited while the action potentials are unaffected. Marmont & Wiersma assume that the action potentials of crustacean muscle are not all or none and that they are not associated with the passage of impulses. They conclude, furthermore, that inhibition may block two processes involved in neuromuscular transmission—the initiation of the muscle action potential and the initiation of the mechanical response.

#### MISCELLANEOUS

*Electrolytes.*—Recent studies, particularly those of Fenn and his collaborators have greatly extended our knowledge of electrolyte equilibria and exchange of ions resulting from activity. Reviews on the earlier work have been given by Fenn (79, 80). As had been previously shown for rats, the leg muscles of the cat lose potassium and gain an equivalent amount of sodium when stimulated (81). In addition there is a gain of water and sodium chloride. Calcium and magnesium are unchanged. The simplest explanation of the interchange of potassium and sodium is the assumption that the muscle fibers shrink during activity, thereby losing some potassium, and that sodium enters only into the inter-spaces. The explanation of the disappearance of sodium during recovery then offers no difficulty. A loss of potassium also occurs during voluntary contraction (swimming of rats). The most enduring animals lost the most potassium and gained the least amount of water (82). In earlier experiments on frog muscle no loss of potassium was observed after stimulation, but Fenn (83) found a considerable loss if the muscles were stimulated at such a rate that the height of the responses was maintained.

From chloride determinations of frog muscle in equilibrium with different chloride concentrations Eggleton *et al.* (84) concluded, in agreement with earlier studies of Fenn, that the chloride of

muscle is dissolved in about one-fourth of the volume of the muscle. It seems certain that no chloride is inside the muscle fibers, but the "chloride space" is probably only partly extracellular space because the connective tissue cells have been shown by Fenn *et al.* (81) to contain chloride.

Fenn & Goettsch (85) showed that during nutritional muscular dystrophy rabbit muscles gain chloride and lose potassium, probably in proportion to the increase of interstitial fluid. This condition resembles degeneration (86) as far as electrolytes are concerned.

*Degeneration.*—Lack of activity leads to degeneration of muscles even with intact motor innervation. In experiments on dogs, Tower (87) cut the dorsal roots of the lumbar region and sectioned the spinal cord anteriorly and posteriorly to that region. The skeletal muscles degenerated although the isolated part of the spinal cord remained alive. However the degeneration following denervation cannot be considered as the result of lack of activity alone because it cannot be prevented by artificially induced activity as shown by several earlier investigators. Furthermore there was no proliferation of the nuclei in Tower's dogs as is typical for the degeneration following denervation. It is concluded, therefore, that an intact nerve supply as well as activity are essential factors for the maintenance of the structure of skeletal muscle.

*Clinical studies.*—The experimental studies on neuromuscular transmission have greatly stimulated the interest in peripheral muscular disorders and remarkable progress in their understanding and treatment has been achieved. An excellent review of this field has been given by Nevin (88). The spectacular effect of eserine and prostigmine in cases of myasthenia gravis discovered by Walker has been confirmed by many investigators (89), but the exact nature of the deficiency is still unknown. It has been suspected that the abnormality of neuromuscular transmission is caused by an increased concentration of choline esterase, but McGeorge (90) found the esterase activity of serum to be within normal limits and Stedman & Russel (91) and Pilcher (92) even report a slightly decreased esterase activity. Because mild curarization produces a condition exactly like that of myasthenia and is similarly influenced by prostigmine and because curare does not affect the liberation of acetylcholine it seems more probable that the threshold of excitation is abnormally raised.

Myotonia, in many respects the reverse of myasthenia gravis,

can be treated effectively by quinine (93). It seems probable that the drug acts by depressing the cholinergic nerve endings. The disease is perhaps caused by a prolonged action of the chemical transmitter.

Familial periodic paralysis likewise is a peripheral disease. During attacks stimulation of the muscles through the skin fails to produce a response. Allot first made the significant observation that the serum potassium is almost invariably lowered during seizures. Aitken and Allot *et al.* (94, 95) and Gammon (96) have extended these studies. It was found that an attack could be prevented or stopped by the administration of 5 grams of potassium chloride. However lower potassium levels than found in these patients produced no symptoms in normal individuals, and no explanation of the special need for potassium has been found as yet. The finding that serum phosphate varies closely parallel with the potassium indicates some disturbance in the muscle metabolism. During the seizures there is a retention of potassium and phosphate, probably an effort at compensation for the low serum potassium and phosphate.

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DEPARTMENT OF PHYSIOLOGY  
OHIO STATE UNIVERSITY  
COLUMBUS, OHIO

## THE DIGESTIVE SYSTEM\*

BY A. C. IVY AND JOHN S. GRAY

*Department of Physiology and Pharmacology,  
Northwestern University Medical School,  
Chicago, Illinois*

The articles reviewed cover the period only from July 1, 1937 to July 1, 1938. The Russian literature for this period has not been available to us, except for a few reprints.

*Salivary secretion.*—Gantt (1) has made a quantitative study of the volume response of the salivary glands to the feeding of various quantities of a standard food to dogs with chronic fistula. A mathematical analysis of 8000 tests revealed a direct linear relationship between the weight of food eaten and the volume of the resulting salivary secretion. An inverse hyperbolic relationship was observed between the quantity of food eaten and the volume of saliva per unit weight of food. The time consumed in eating was found to have no significant effect on the response of the salivary glands. These results were contrasted in a second report (2) with those obtained from an analysis of the conditioned reflex response of the glands. A direct logarithmic relationship was found between the volume of saliva secreted in response to a conditioned reflex and the quantity of food administered during the conditioning process. This relationship recalls the Weber-Fechner law governing the ratio of intensity of stimuli to the intensity of sensations.

Deutsch & Raper (3) in an investigation of the metabolism of isolated slices of cat salivary glands have found that a limited quantity of substrate is present which may serve for oxidation either in rest or during activity stimulated by the addition of acetylcholine and eserine. The R.Q. under these conditions varied between 0.59 and 0.8, and iodoacetate or fluoride ions did not abolish oxidation, which indicated that the substrate consisted of more than glucose. The addition of acetylcholine rapidly exhausted the substrate, after which the drug failed to produce its usual acceleration of oxygen consumption. If glucose was added at this time, a maximal stimulation with acetylcholine was obtained, accompanied by an R.Q. of 1.0. This action of the drug could be abolished by iodoacetate or fluoride, by atropine, or by the exclusion

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of oxygen. During activity the salivary glands preferentially oxidized glucose. The gland tissue differed from muscle tissue in that it was incapable of accomplishing anaërobic metabolism.

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Langstroth, McRae & Stavraký (4) have applied spectroscopic methods to the chemical analysis of saliva secreted at various rates in cats in response to stimulation of the chorda tympani nerve. They report that a direct linear relationship exists between the sodium concentration of saliva and the volume-rate of secretion, whereas the potassium concentration remains constant and independent of the volume-rate. At very low rates of secretion, however, the concentrations of both sodium and potassium increase, revealing a significant degree of absorption of water at these low rates. The columnar epithelium of the striated tubules of the salivary duct system consists of cells which resemble those of the proximal convoluted tubules of the kidney; perhaps it is these cells that are responsible for this reabsorption of water. The protein concentration of the saliva also increased with the rate of secretion, but as the period of stimulation continued its concentration tended to fall as a result of exhaustion of its intracellular precursors. An equation was developed mathematically, which predicted the behavior of the proteins from the behavior of the metallic ions. From this equation it was concluded (a) that membrane permeability and the secretion of water and protein all depend upon the rate of liberation of an activating substance within the cell in response to nerve stimulation, and (b) that the mechanism of protein secretion consists of a chemical reaction which transforms granule material to a form readily carried out of the cell by a flow of water.

The work of Wills & Fenn (5) on the potassium balance of the salivary glands of cats demonstrates a linear relationship between the intake and output of potassium during secretory activity resulting from electrical stimulation of the chorda tympani or from the administration of small doses of pilocarpine. With larger doses of the drug, however, the intake of potassium fell off. They obtained a reduction of 4 mg. per 100 cc. in the potassium level of the blood during passage through the glands, whether stimulated through the nerve or by pilocarpin, in spite of the fact that the rate of blood flow was much greater during nerve stimulation.

Keszytus & Martin (6) have compared the composition of saliva produced by chorda stimulation with that produced by sympathetic stimulation in dogs. They found that sympathetic saliva is higher in potassium, calcium, organic solids, mucin, and albumin, and lower in sodium and chloride than chorda saliva. During stimulation of either nerve the concentrations of the various constituents decreased as the period of secretion was extended. In view of the above-mentioned evidence of Langstroth, McRae & Stavrakys (4) and of others that the composition of saliva is a function of its rate of secretion, it is difficult to interpret the results of experiments in which this factor is not taken into consideration.

Pierce & Gregersen (7) have compared the response of dogs with chronic salivary fistula to the continuous intravenous injection of pilocarpine before and after unilateral section of the chorda tympani. The response to pilocarpine was unchanged on the intact side, but contrary to their expectations the response of the denervated gland was augmented, reaching after two to three weeks high levels, which persisted for at least one year. The augmented response was not due to the accumulation of preformed saliva in the duct passages, nor to increased blood flow. They did not attribute it to the specific sensitization produced by denervation, since acetylcholine and acetyl- $\beta$ -methylcholine gave a greater response on the normal than on the denervated side. However, these latter drugs were injected at enormously greater rates than the pilocarpine, and they are known to have an optimal secretory dosage. The possibility should be considered that the optimum was exceeded to a greater extent in the gland sensitized by denervation, than in the normal gland. However, the explanation may not be as simple as this, for Simeone & Maes (8) have reported that removal of the cervical sympathetic ganglia in the cat, sensitizes the salivary gland to epinephrine and also to pilocarpine, but not to acetylcholine.

Gibbs & McClanahan (9) have reported that histamine, in contrast to acetylcholine, produces an irregular secretory response in the salivary gland when injected in minute doses directly into the arterial supply of the gland. The stimulating effect of histamine, when present, was potentiated by eserine and abolished by atropine. Histamine was also found to potentiate the effect of chorda stimulation, but not that of acetylcholine. They conclude

from these observations that histamine may act by permitting a continuation of the process initiated by nerve stimulation, *i. e.*, it may remove a natural "brake" mechanism. Such a concept, if established, might be of significance in understanding paralytic salivary secretion.

Meyer, Golden, Steiner & Necheles (10) have reported that the ptyalin content of saliva is greatly reduced in old age.

*The stomach.*—Bussabarger, Freeman & Ivy (11) report that when the stomach is removed from growing puppies maintained on an adequate diet, the bones fail to ossify normally. Homogeneous osteoporosis is so severe that bony deformities and even spontaneous fractures result. The osteoporosis is analogous to that observed clinically in severe cases of celiac disease without rickets. The deficient ossification is apparently due to a combination of at least three factors: the absence of hydrochloric acid which normally renders the less soluble calcium salts more soluble and assists in the maintenance of an acid reaction in the intestine; the absence of the reservoir function of the stomach which results in an increase in the rate of intestinal transport; the presence of a post-cibal acidosis which is unfavorable for calcium retention.

Petri, Norgaard & Bing (12) have reported that gastrectomy in growing pigs results in severe nutritional disturbances characterized by anemia, skin and spinal cord changes, osteoporosis, and extreme emaciation. They maintain that the syndrome resembles human pellagra. The difference in the results obtained by Bussabarger, Freeman & Ivy and those obtained by Petri, Norgaard & Bing may probably be attributed to the relative adequacies of the diets used.

Jemerin & Hollander (13) conclude from their anatomical study of the distribution of the vagus nerves to the stomach in the dog that in preparing the ordinary Pavlov pouch as much as 75 per cent of the vagus fibers to the pouch may be interrupted.

*Gastric secretion.*—Gersh (14) has applied his freezing-drying histological technique to the study of the distribution of chloride in the gastric glands of dogs. At the height of secretion the animals were exsanguinated under ether anesthesia and a small piece of gastric mucosa was separated from the muscularis and frozen in liquid nitrogen. After desiccation in a vacuum at  $-60^{\circ}\text{C}$ . tests for chlorides were made with silver nitrate. No chloride was found in the cytoplasm of any of the gland cells, although it was detected

in the zymogen granules of the chief cells and along the border of the lumen and foveolus. Active glands contained more chloride, but the distribution was identical with that of resting glands. Two possible explanations were offered for the absence of chloride in the cytoplasm of the parietal cell. A non-hydrolysable form of chloride could not be detected by the method and might, therefore, be postulated, but this would be incompatible with Bensley's theory that the bound chloride secreted by the parietal cell is freed by the simple process of dilution hydrolysis. An alternative explanation is that the chloride is secreted from the cell as rapidly as it enters. A third possibility that might be considered is that during the process of bleeding under ether anesthesia, and during dissection of the tissues the previously active cells have reverted to the resting state. The author was interested only in the distribution of chlorides, but since desiccated tissue obviously could not retain hydrogen chloride gas, the procedure used could only detect neutral chloride. It is questionable whether such a technique could yield any information concerning the mechanism of hydrochloric acid formation.

Quigley, Barcroft, Adair & Goodman (15) have investigated the claims that secretory activity of the gastric glands is accompanied by changes in electrical potentials of the gastric mucosa. By recording the difference in potential between the inner surface of the stomach or pouch, and the skin in dogs with a Pavlov pouch, they could detect no changes accompanying either gastric secretory or motor activity. However, the potential of the pouch as well as of the main stomach was lowered by introducing milk, dextrose, and alcohol into the main stomach, but not by intravenous administration. Emulsified fat in the main stomach had no influence on the pouch potential, which eliminated enterogastrone as the agent responsible for the depression of potentials. The potential depended, however, on some unknown "vital" activity, since it was abolished by the intravenous injection of sodium cyanide.

Although atropine has never been shown to abolish histamine secretion, it does abolish the gastric secretory response of dogs to a meal. This has constituted an objection to the theory that histamine is gastrin, although the effects of atropine on a meal stimulus and histamine stimulus of equal potency have not been compared. Gray (16) has shown that atropine completely abolishes the limited secretion of the total pouch dog to a meal, but that it

can only partially inhibit a secretion of the same rate produced by repeated injections of small doses of histamine. Accordingly it was concluded that either histamine is not gastrin, or atropine must prevent its formation.

Using Barsoum & Gaddum's method for the determination of blood histamine, MacIntosh (17) was unable to detect any increase in histamine in dog's blood during digestion of a meal. However, histamine was found in gastric juice in higher concentration than in the blood both after sham feeding and after histamine injections. The concentration of histamine increased with the rate of secretion of juice. Since the histamine concentration of the blood remained unchanged on passing through the stomach, and since the blood histamine is considered to be bound in the formed elements and not free in the plasma, it was concluded that histamine is formed in the gastric mucosa and secreted by the parietal cells. The hypothesis was advanced that histamine mediates the secretion of the parietal cells. Babkin (18), discussing these findings, believes that acetylcholine liberated by the vagus nerve in turn liberates histamine which stimulates the parietal cell. Thus, histamine is considered to be involved in the normal secretory process of the parietal cell, but not as the hormone, gastrin.

Komarov (19, 20) claims that extracts of the pyloric mucosa, which are free of histamine, stimulate gastric secretion on intravenous injection. The largest amounts of potent material were obtained from the pylorus, smaller amounts from the intestine, and none from the fundus. The action of this gastrin preparation is reported to be unaffected by atropine.

MacIntosh & Krueger (21) have attempted to evaluate the importance of choline as a secretagogue mediating the chemical phase of gastric secretion. They found choline to be a weak stimulus for the secretion of fluid and hydrochloric acid, but a strong stimulus for the secretion of pepsin. They also report that the response to a meal is augmented by administration of choline even after its direct secretory effect has worn off. The quantities of choline in the diet indicate it to be of little importance as a secretagogue, except by its possible delayed secondary effect. They noted a greater response to choline when injected into the jugular vein than when injected into the superior mesenteric vein, presumably because of the ability of the liver to remove it from circulation. Gerez & Weiss (22) have shown that the establishment of an

Eck fistula in dogs with a Pavlov pouch markedly increases the volume, acidity, and duration of secretion, whereas the pepsin content is decreased. This work provides further evidence that the liver removes secretogogic substances from the blood.

Wilhelmj, McCarthy & Hill (23) have continued their investigations of the importance of the acidity of the gastric and duodenal contents for the regulation of the acidity of the total secretions in the stomach. It had previously been shown that the presence of high acidity in the stomach inhibits the gastric phase of secretion, and that high acidity in the intestine inhibits the intestinal phase of secretion. They have not reported that the cephalic phase is sufficiently intense to overcome the inhibitory effect of high gastric and intestinal acidity. The contributions of this group to the problem of the regulation of gastric acidity have been recently summarized (24). It is perhaps unfortunate that the phenol-red method of determining the degree of dilution of test meals by the gastrointestinal secretion, as adapted by this group, gives no information in regard to the quantities of juices secreted. It merely reveals what proportion of an unknown volume of gastric contents is made up of secretion, and what proportion consists of the test meal. The significance of this important work would be still greater if the actual quantities of fluid secreted or regurgitated into the stomach were determined, perhaps by such a modification as that devised by Bloomfield & Keefer (25).

It has been shown that fat inhibits gastric secretion and motility by a humoral mechanism (enterogastrone). Although it has also been shown that glucose inhibits gastric motility by the same mechanism, there has been little evidence that glucose is capable of inhibiting the secretory activity of the stomach. Manville & Munroe (26) have reported that washing the stomach with hypertonic glucose solution decreases the acidity of the washings during gastric secretory activity stimulated by histamine, pilocarpine or insulin.

To the still unsettled question as to whether histamine stimulates pepsin secretion, Rivers & Vanzant (27) have contributed their results obtained by the use of the double histamine test in man. They report that when histamine is given in two injections, one hour apart, analysis of the continuously collected gastric juice reveals as great a pepsin output in response to the second injection as to the first. They consider that washing out of preformed pepsin



from the gastric tubules can not account for these findings and that histamine must therefore stimulate the secretion of pepsin.

x Loman, Rinkel & Myerson (28) report that administration of acetyl- $\beta$ -methylcholine by iontophoresis produces a flow of alkaline gastric juice in human subjects. Gray & Ivy (29) have shown that when small doses of acetyl- $\beta$ -methylcholine (0.1 mg. or less) are injected subcutaneously every ten minutes into dogs with pouches of the entire stomach, a copious flow of highly acid gastric juice is obtained. However, larger doses are much less effective. In contrast to histamine, acetyl- $\beta$ -methylcholine will not maintain a prolonged uniform flow of secretion. This evidence that the effect of acetyl- $\beta$ -methylcholine might be reversed with increasing dosage was confirmed by administering large and small doses to dogs while secreting continuously and uniformly to repeated injections of histamine. Doses of 0.1 mg. of acetyl- $\beta$ -methylcholine markedly augmented, whereas doses of 1.0 mg. markedly diminished the histamine secretion. Both of these effects could be abolished by atropine. This reversal of effect with dosage is probably of significance in interpreting the contradictory reports in the literature in regard to the effect of choline derivatives in gastric secretion. Necheles, Motel, Kosse & Neuwelt (30) reported that in dogs with Haidenhain pouches, acetyl- $\beta$ -methylcholine in doses greater than the optimum as determined by Gray & Ivy, first inhibited, then stimulated the secretion of acid, water, and pepsin. Large doses were found to depress the secretion stimulated by histamine and pilocarpine. In human subjects they found that acetyl- $\beta$ -methylcholine increased the total volume of juice and stimulated the secretion of acid and pepsin, but if the subjects were permitted to swallow the profuse saliva, the acidity was neutralized. Schnedorf & Ivy (31) reported that monkeys which failed to respond to histamine injections (seventeen per cent of monkeys) did respond to acetyl- $\beta$ -methylcholine; following this they became responsive to histamine.

Loman, Rinkel & Myerson (28) report that benzedrine by iontophoresis causes a slight increase in intragastric acidity. Smith & Chamberlin (32) find that benzedrine either has no effect or slightly increases gastric acidity during a test meal. According to Rafferty, Van Liere & Sleeth (33) ephedrine by mouth decreases the acidity response to a test meal in human subjects. Atkinson & Ivy (34, 35) have shown that ergotamine tartrate in large doses,



as well as various emetics, produces a diminution in the secretory response of the Pavlov pouch dog to a meal and to histamine. Theelin, emmenin, larostidin, pituitrin, pitressin, and certain chemical compounds were without effect. Dionessov (36, 37) has shown that both epinephrine and pituitrin inhibit gastric secretion in Pavlov pouch dogs. Although ergotamine tartrate abolished the inhibitory effect of pituitrin it had no effect on the action of epinephrine. He concludes, therefore, that the action of epinephrine is directly on the gastric gland cells, whereas that of pituitrin is indirect through its vasoconstrictor properties. According to Pickett & Van Liere (38) anoxemia produced by low pressures equivalent to an altitude of 1400 to 1800 feet depresses acid secretion in the Pavlov pouch dog.

Espe & Cannon (39) have shown that calves do not have a psychic phase of gastric secretion. This is in keeping with their eating habits. Kurzin & Slupsky (40) have obtained a copious flow of gastric juice in human subjects in response to mechanical stimulation of the stomach. Hardy & Quanstrom (41) have found that more secretion can be removed from the dog's stomach after obstruction of the cardia and jejunum when the gastric contents are drained intermittently than when the contents are allowed to accumulate. Streicher, Snyder, Liebman & Keeton (42) have shown that patients with achylia gastrica do not show a volume response to injections of histamine. A study of the chemical composition of the gastric juice revealed that the parietal cells had made no contribution to the secretion. By means of the Linderström-Lang technique, Glick (43) has localized the choline esterase content of the gastric mucosa in the epithelial cell region. The amount of choline esterase was not influenced by the physiological state of the animals, or by the administration of acetylcholine, eserine, or atropine. Bloch & Necheles (44) have reported that human and canine gastric juices contain no choline esterase, but do contain acetylcholine, Euler's P substance, and a third pharmacologically active, but unidentified compound.

Thompson (45) has reported that a potent anti-pernicious anemia preparation can be made from desiccated duodenal mucosa of hogs as well as from the stomach. This confirms the view that the "intrinsic" factor of Castle is not elaborated solely by the gastric mucosa. However, Magnus & Ungley (46) have found that the stomachs of patients with pernicious anemia show at autopsy

a severe atrophy of all the layers of the stomach wall, but that this atrophy is strictly limited to the fundic portion. Failure of production of the "intrinsic" factor in pernicious anemia is therefore not the result of atrophy of the mucosa. Taylor, Castle, Heinle & Adams (47) claim that normal gastric juice, in contrast to gastric juice obtained from patients with pernicious anemia, possesses proteolytic activity not due to pepsin and therefore presumably due to the "intrinsic" factor. Jones, Grieve & Wilkinson (48), on the other hand, believe that the proteolytic activity of desiccated mucosa may be due to pepsin. Castle, Heath, Strauss & Heinle (49) suggest that the "intrinsic" factor may operate not in the gastrointestinal tract, but parenterally, since they find that it is inactive at acidities below pH 2.5. They have also shown, contrary to previous opinions, that incubation of the "intrinsic" and "extrinsic" factors does not result in the formation of the thermostable principle of the liver. Helmer & Fouts (50) and Goldhamer & Kyer (51) have published methods for obtaining concentrated preparations of the "intrinsic" factor.

*Gastric motility.*—The factors involved in regulation of gastric evacuation have received considerable attention in the recent literature. Meschan & Quigley (52) have studied the behavior of the gastric outlet by means of three tandem balloons located in the pyloric antrum, the pyloric sphincter, and the duodenal bulb in trained unanesthetized dogs. Their results show that these three regions operate as one functional unit; waves which originate in the stomach pass successively over all three structures. The pyloric sphincter tends to be passively relaxed most of the time, contracting only when a peristaltic wave reaches it. This contraction persists while the wave proceeds over the duodenal bulb. The sphincter therefore operates to prevent regurgitation to a much greater extent than to regulate evacuation. This complex, coordinated behavior of the gastric outlet, is not disturbed by vagotomy. Quigley & Meschan (53) have also reported that many substances, when introduced into the duodenum, affect the motor activity of the gastric outlet, but that each of the three structures composing it is influenced in the same direction. There was no indication of antagonistic effects on the viscus and sphincter as is so generally presumed to occur. The following substances in descending order of effectiveness inhibited motility in the pyloric region: fats, fatty acids, dextrose, soaps, dilute mineral acids, hypertonic salt solu-

tions, glycerol, and hypotonic salt solutions. In chronic bilaterally vagotomized animals the inhibitory effects of these materials were greatly decreased or abolished, indicating that they operate mainly through the enterogastric reflex.

Thomas (54) has employed a different technique in the analysis of events occurring in the gastric outlet during evacuation. By means of a differential manometer, the differences of pressure between the duodenal and gastric sides of the pyloric sphincter were recorded in fistula dogs. When the stomach contained food, the intragastric pressure was generally maintained at a slightly higher level than that in the duodenal bulb, even during periods of relative inactivity. Cyclic changes in pressure were observed which consisted of first a rise in intragastric pressure over intraduodenal pressure, followed by a return of the intragastric pressure to that of the duodenum, and finally a rise in intraduodenal pressure over that of the stomach. This cycle presumably represented the passage of a peristaltic wave over these structures. Incomplete cycles were commonly observed, the most frequent of which lacked the initial augmented intragastric pressure. This incomplete cycle was characteristic of the stomach when pure water was being rapidly evacuated. The complete cycle was most often observed during evacuation of solid foods. Introduction into the duodenum of hydrochloric acid or peptone, which elicit the enterogastric reflex, produced a prompt equalization of the pressure on the two sides of the sphincter, indicating relaxation of the latter structure. More direct proof that the pyloric sphincter plays only a secondary rôle in the regulation of normal gastric evacuation has been presented by Crider & Thomas (55). They could detect no change in the emptying time of isotonic saline, 5 per cent glucose, 0.05 *N* hydrochloric acid, 10 per cent alcohol, or olive oil in dogs when the pyloric sphincter was kept open continuously by means of a special perforated tube.

Crider & Thomas (56) have analyzed those factors which regulate gastric evacuation by elicitation of the enterogastric reflex from the duodenum. They report that proteoses and peptones are active in this way when introduced into the duodenum. Protein fractionation of intestinal contents by means of ammonium sulphate revealed that approximately one half of their activity was attributable to proteoses, and one half to peptones and other substances. Acidity was found to be a minor factor at pH's above 3.0.

The conclusion was drawn that in the normal regulation of gastric evacuation, acidity of the chyme is of much less importance than the digestion products of the food.

Gershon-Cohen & Shay (57) have shown in man that the presence of hypertonic solutions in the intestine markedly delays gastric evacuation. In a previous paper (58) it was claimed that this delay is due to spasm of the pylorus, rather than to inhibition of gastric peristalsis. The proof offered in support of this claim will not, in the reviewers' opinion, withstand critical examination, yet the significant point appears to have been made, that a water-barium mixture may be emptied rapidly from the stomach, particularly in achlorhydric subjects, without evident peristaltic activity. It was mentioned above that Meschan & Quigley found the pylorus to be usually "relaxed" in the empty stomach. Thomas found the pyloric sphincter to be "relaxed" during evacuation of water, as revealed by the absence of pressure rises in the stomach. However, when the stomach contained food the sphincter must have been usually "contracted" (closed), because intragastric pressure was maintained at a higher level than that in the duodenum. Perhaps these results may be interpreted in the following way: when food is eaten, the first portion entering the duodenum may, if sufficiently stimulating, temporarily close the pylorus by Thomas' myenteric reflex, and very likely does elicit the enterogastric reflex, which relaxes the stomach and retards evacuation; this permits food to accumulate in the stomach, which may then be responsible for raising the pyloric tonus, just as it raises the cardiac tonus; this slightly increased tonus is sufficient to prevent evacuation, in the absence of peristalsis. Thus, water may be rapidly evacuated from the stomach through a relaxed sphincter, without peristalsis, but food which accumulates in the stomach increases pyloric tonus and requires peristalsis to aid in its evacuation. Strong duodenal irritation, as that produced by duodenal instillation of hydrochloric acid or hypertonic solutions, etc., will markedly relax both the stomach and the sphincter, thus delaying emptying and promoting regurgitation. It would appear that Quigley's and Thomas' interpretation minimizes the importance of the pyloric sphincter and fails to explain the evacuation of water without peristalsis while, on the other hand, Gershon-Cohen & Shay's interpretation greatly exaggerates the importance of the pylorus and fails to explain any of the experimental findings in dogs.

Brücke & Stern (59) have investigated the innervation of the cardiac sphincter in cats from the standpoint of locating the adrenergic and cholinergic fibers and their functions. The tonicity of the sphincter was measured not by a balloon, but by the height of a column of water which the sphincter could support. Bilateral vagotomy produced a cardiospasm which was abolished by atropine and epinephrine but exaggerated by eserine, acetylcholine, and pilocarpine. Stimulation of the peripheral end of the vagus, or stimulation of the central end of one vagus with the other intact, produced relaxation of the sphincter. The vagus was therefore shown to be inhibitory to the sphincter, an effect of adrenergic fibers since the action could be potentiated by cocaine and abolished by ergotamine. Stimulation of the splanchnics after vagotomy (to eliminate reflex opening) and denervation of the adrenals (to eliminate epinephrine production) produced cardiospasm. The effective fibers were shown to be cholinergic, since the action was potentiated by eserine, and abolished by atropine. Brücke & Stern in their discussion make the following observation. The fact that previous investigators had been able to observe the inhibitory effect of the vagus on gastric motility only after abolishing the excitatory effect with atropine, is related to the fact that the initial gastric tonus was always low; had the tonus been augmented with eserine or pilocarpine the inhibitory effect of the vagus would have been revealed without the use of atropine. These findings explain the well-known observation that the vagus sometimes relaxes and sometimes contracts the stomach according to the tonus level, without requiring the hypothesis that a given nerve fiber can produce opposite effects.

Gayet, Minz & Quivy (60) have reported that stimulation of the splanchnics in vagotomized, atropinized, and eserinizied dogs with the adrenals removed liberates acetylcholine into the venous blood from the stomach with regularity, from the small intestine inconstantly, and from the pancreas inappreciably. This work again emphasizes the importance of classifying autonomic nerves, and interpreting their functions on the basis of the chemical mediator liberated rather than on their anatomic origin. These nerves are frequently mixed ones, and crude electrical stimulation which activates an entire nerve trunk produces variable results which may be quite misleading. In normal physiological processes an entire nerve trunk is probably rarely activated; only those fibers

are chosen which produce the "proper" response, as witness the invariable gastric inhibition mediated by the mixed vagus nerve in the enterogastric reflex and the "receptive relaxation" of the stomach after swallowing.

For the same reasons the interpretation of the effects of section of mixed nerves is not always simple. Barron (61) has summarized his observations on the effects of splanchnicotomy and vagotomy on human gastric motility. He found that section of the splanchnics increased the motility of the empty stomach, as recorded by the balloon technique, both in regard to the duration of periods of activity and the amplitude of contractions. Evacuation time as determined by roentgenological methods was significantly shortened. In a "vagotonic" patient with hypermotility, delayed evacuation, and "pylorospasm," section of the vagi reduced the motility of the empty stomach, but hastened evacuation of a barium meal. The interpretation of these results is complicated by the evident pathological behavior of the stomach of this patient prior to operation, but it is conceivable that the improvement in evacuation time reflects the removal of duodenal regulation of gastric evacuation, whereas the reduced motility of the empty stomach is the result of removal of the excitatory functions of the vagus. The motility of the empty stomach should not be confused with the motility of the emptying stomach, for the two need not necessarily be influenced similarly by all procedures.

Hermann, Morin, Jourdan & Vial (62) report that removal of the dorsolumbar and sacral cord, which eliminates the sympathetic outflow to the stomach, hastens gastric evacuation in dogs. The delay in evacuation resulting from bilateral vagotomy in one dog, persisted after removal of the cord, although emptying was quite variable because of periodic attacks of anorexia and meteorism. This animal died on the one hundredth day with a chronic gastric ulcer.

Derbyshire & Ferguson (63) have uniformly produced reflex vomiting in decerebrate dogs and cats by stimulation of the central end of the vagus. The cardia was observed to open several seconds before retching began. Recovery of excitability of the reflex occurred within one minute in dogs and within from two to ten minutes in cats. Ether, dial, or oxygen-lack depressed the reflex.

Youmans & Meek (64) have reported what appears to be a new type of physiological mechanism for accomplishing gastric and

intestinal motor inhibition. They found that mechanical stimulation of the rectum and anus in dogs inhibited gastric and jejunal motility and tone after vagotomy and removal of the adrenal medullae. The effect was abolished by splanchnic section and chain ganglionectomy, but a humoral mechanism was revealed by the fact that the completely denervated jejunum continued to respond after vagotomy and demedullation of the adrenals. They conclude that mechanical stimulation of the colon liberates a humorally acting inhibitory substance from the sympathetic nerve endings of the colon.

Lalich, Youmans & Meek (65) have confirmed the fact that insulin has a stimulatory effect on gastric motility mediated by the vagi, but have reported that vagotomy not only abolishes the excitation, but converts it to inhibition. They suggest that insulin has a peripheral inhibitory action. In support of this interpretation Farah (66) has shown that insulin relaxes isolated strips of rabbit small intestine. However, crystalline insulin was somewhat less potent than cruder preparations.

Beyer & Meek (67) have reported that benzedrine sulfate produces a 30 per cent reduction in the initial emptying time, and a slight delay in final emptying of the stomach in dogs. In explanation of these results they found that benzedrine first augmented then, after forty minutes, completely inhibited motility of the empty stomach. Beyer (68) further showed that benzedrine temporarily inhibited activity but increased the tonus of the pyloric sphincter, followed by a return to a slower, more regular motility of greater amplitude. Van Liere & Sleeth (69) and Smith & Chamberlin (32) have reported that benzedrine delays gastric evacuation in man.

Although atropine has again been shown to reduce gastric motility (70, 71, 72), Veach, Lauer & James (73) have reported that a previous injection of prostigmin which inhibited the stomach converted the action of atropine to a stimulation of motility. The effects of the drugs were reversed in their actions on the colon. One wonders whether the paradoxical effects on the stomach might not be due to reflex effects from the colon or other portions of the body.

Levina (74) reports that pituitrin in adequate doses delays evacuation through a gastric fistula in dogs. Sleeth & Van Liere (75) have found that chloroform and ether, which most frequently produce ileus, depress gastric motility more than ethylene, cyclo-



propane, and divinyl oxide. Marcus & Necheles (76) claim that estrus and early pregnancy definitely prolong emptying time. Barden, Ravdin & Frazier (77) have pointed out that, after partial gastrectomy, a low-protein diet leading to a nutritional edema produces a delay in gastric evacuation in man and in dogs because of swelling of the tissues at the site of the artificial outlet to the stomach. Barron (78) claims that although posterior gastroenterostomy does not affect motility of the empty stomach, partial gastrectomy increases the amplitude of contraction. No statement is made in regard to adjustment of the size of the recording balloon to the size of the operated stomach.

*Peptic ulcer.*—Two papers have recently appeared which again emphasize the unimportance of hyperacidity in the etiology of peptic ulcer. Schmidt & Fogelson (79) sham fed dogs ten to twelve hours a day for as long as 102 days without obtaining the least evidence of chronic ulcers. Brown & Dolkart (80) have followed the gastric acidity of a series of patients through repeated recurrences of ulcer symptoms. They found no significant trend in gastric acidity, either prior to or after recurrences, nor could they detect a correlation between the acidity and the degree of distress.

Martin & Schnedorf (81) have made an extensive investigation of the claims that lesions of the hypothalamus produce peptic ulcers. In seven monkeys and forty cats localized lesions were placed in various portions of the hypothalamus by means of the Horsley-Clark stereotaxic instrument. No changes were noted in gastric secretion or motility, and neither bloody stools nor vomitus were ever observed. Eventual autopsy of the animals revealed no pathological changes in the gastrointestinal tract.

Stalker, Bollman & Mann (82) have reported that peptic ulcers can be produced uniformly in animals by the oral, rectal, and par-enteral administration of cinchophen. This work eliminated a local effect of the cinchophen on the gastric mucosa. The course of development of the ulcers resembled that seen in man.

Since peptic ulcers occur only rarely in pregnancy, and when present show a tendency to remission, Sandweiss, Saltstein & Farbman (83) have studied the effect of sex hormones on the incidence of ulcers in dogs with a Mann-Williamson operation. Twelve control animals as well as fifteen animals treated with estrone died with peptic ulcers. Of fifteen animals treated with pregnancy-urine extract (antuitrin-S) only seven developed ulcers, and

of these four showed evidences of healing. There is as yet no explanation for the action of the extract.

Two theories of the etiology of peptic ulcer have recently been advanced. Necheles (84) attributes to liberated acetylcholine, the role of producing vasoconstriction in the stomach, thereby devitalizing the tissues and rendering them susceptible to the digestive action of hydrochloric acid and enzymes. Babkin (18) attributes a similar role to histamine.

*Intestinal secretion and motility.*—Nasset (85) has published a method for the preparation of enterocrinin, a duodenal hormone which stimulates the secretion of succus entericus. The extracts are claimed to be free of vasodilatin, and to have no secretin action on the pancreas. Enzyme production as well as volume of secretion is stimulated in the intestinal glands. Nasset, Schriffirin & Belasco (86) have further reported that the presence of hydrochloric acid in the duodenum does not liberate enterocrinin as it does secretin. Enterocrinin extracts were found to augment the oxygen consumption of isolated slices of intestinal mucosa. The extracts are without effect on the blood-sugar level of normal animals, hence they do not contain LaBarre's "incretin" or Heller's "duodenin".

Hukuhara & Kinose (87) have investigated the existence of a humoral mechanism for the regulation of intestinal motility. In dogs under morphine-ether anesthesia a segment of intestine was isolated and its nerve supply completely interrupted. The introduction of 0.4 per cent hydrochloric acid into the duodenum elicited a flow of pancreatic juice, but had no effect on the motility of the isolated segment of intestine. These results might be objected to on the grounds that the etherized animal is not an ideal animal in which to study intestinal motility and that hydrochloric acid need not be the substance which causes the liberation of the supposed hormone.

In regard to the possible role of the duodenum in the humoral regulation of carbohydrate metabolism, Shay, Gershon-Cohen & Fels (88) have contributed some interesting results obtained on human subjects. By instillation of dilute acids, hypertonic solutions, or fat into the duodenum, glucose solutions introduced into the stomach are completely prevented from being evacuated. Since the glucose recoverable from the stomach under these conditions is less than that originally introduced, Shay & Gershon-Cohen

concluded that some glucose was absorbed from the stomach. However, this was not accompanied by a rise in blood sugar. If the same quantity of sugar in isotonic solutions was introduced into the intestine without duodenal irritation with acids or fat, a definite hyperglycemia occurred. No effect on the fasting blood-sugar level or epinephrine hyperglycemia could be demonstrated when the duodenum was stimulated with acids, but a reduction of a diabetic hyperglycemia was obtained in one patient.

In regard to the effect of benzedrine on intestinal motility, Beyer (68) reports it to have no constant effect; Smith & Chamberlain (32) claim that it reduces intestinal motility, and Boyd (89) reports that it produces a spastic contraction of isolated uterine and intestinal strips.

Abderhalden & Abderhalden (90) report that isolated intestinal strips of pigeons on a thiamin deficient diet, are resistant to the contracting effect of acetylcholine, and that the addition of thiamin to the bath lowers the threshold to acetylcholine without in itself producing a contraction. Chabrol, Lemaire & Cottet (91) report that intravenous injections of bile salts produce an evanescent decrease in intestinal tone in chloralosed dogs. Continuous intravenous administration produces a complete paralysis, with failure to respond to acetylcholine or epinephrine. In a patient with exteriorized bowel, Forster (92) has shown that morphine increases non-propulsive motility and decreases propulsive motility. According to Burstein (93) cyclopropane, in contrast to ether, increases intestinal motility, although deep anesthesia may inhibit.

*Intestinal absorption.*—In regard to the role of the leukocytes in the absorption of fat from the intestine, Leach (94) claims that neither the lymphocytes nor eosinophils of the intestinal mucosa contain fat particles. Although the phagocytic cells were found to contain fat granules, the available evidence indicates that they are not migrating away from but toward the intestinal lumen. Benzene poisoning in rabbits retarded fat absorption and reduced the blood lymphocyte count, but the lymphocytes of the intestinal mucosa were unaffected except for pyknotic nuclear changes. Leach concludes that there is no evidence to suggest that leukocytes aid in the transport of fat across the intestinal epithelium.

In regard to the theory of Verzář that bile salts aid in fat absorption by rendering the fatty acids more diffusible, Breusch (95) has recently claimed that the higher unsaturated fatty acids, when

combined with bile salts, do not diffuse through artificial membranes. Vonk, Engel & Engel (96) have developed two methods by which fatty acids may be determined in the presence of bile acids. Using these methods they also were unable to demonstrate diffusion of higher fatty acids through artificial membranes. It is possible, of course, that the membranes used by the various workers have not had identical permeabilities, but it is also true, that the fact that fatty acid-bile complexes can or cannot diffuse through artificial membranes is very unsubstantial evidence for their diffusibility through the intestinal mucosa.

Some years ago Sinclair (97), later confirmed by Artom & Peretti (98), demonstrated a rapid turnover of phospholipids in the intestinal mucosa of fed animals. Artom *et al.* (99) have recently demonstrated this phenomenon again by feeding radioactive "labeled" phosphorus. Perlman, Ruben & Chaikoff (100) have used "labeled" phosphorus to demonstrate the rapid turnover in the fasted animal. According to Fries, Ruben, Perlman & Chaikoff (101) the small intestine only is involved in this process.

It has not been settled whether this rapid turnover indicates that the phospholipids are intermediary products in the resynthesis of absorbed fatty acids and glycerol, or whether it indicates that phospholipids are synthesized in the intestinal mucosa and then distributed to the rest of the body through the blood stream. Sinclair & Smith (102) incline to the latter interpretation and, as a result of experiments on feeding unsaturated fats and the chemically identifiable elaidic acid, they conclude that in the process of synthesis there is a selection of one molecule of a saturated fatty acid for each molecule of an unsaturated acid. In favor of the view that phospholipids are synthesized in the mucosa and distributed by means of the blood stream is the fact that a phospholipidemia follows the feeding of fats. However, Hevesy & Lundsgaard (103) have fed olive oil and "labeled" phosphorus to animals and were unable to show that the increased phospholipids of the blood contained the "labeled" phosphorus.

Frazer (104, 105, 106) has shown quite clearly that the ultra-microscopic particles visible in blood under dark-field illumination are particles of fat with a layer of globulin adsorbed on the surface. He claims that particle counts provide a simple and rapid method of comparatively determining the fat content of blood. By means of this method he has shown that when rats are fed olive oil the

particle count in the systemic circulation rises, whereas the particle count in the portal blood decreases. When a mixture of fatty acids and glycerol is fed, however, the situation is reversed, the particle count in the systemic circulation falls and in the portal blood rises. It was also shown that when rats were fed on fats stained with Sudan III, the fat depots became stained with the dye, but not the liver; on the other hand, when stained fatty acids and glycerol were fed, the liver showed signs of the stain and the depot fat did not. Frazer concludes from this that that portion of the dietary fat which is hydrolyzed forms soluble complexes with bile salts and is absorbed into the portal circulation to be stored by the liver; the remainder of the dietary fat is absorbed without hydrolysis into the lymphatic system and is discharged into the general circulation to be stored in the fat depots.

It has been known for several years that patients with sprue, non-tropical sprue, and celiac disease show a minimal rise in blood sugar on ingestion of glucose (107). Barker & Rhoads (108) have recently shown that patients with sprue do not show the normal increase in blood fats following the ingestion of a fatty meal. These results support the view that absorption is seriously impaired in these diseases. Following the administration of liver extract both the disease and the abnormal glucose and fat-tolerance curves are improved. Castle, Rhoads, Lawson & Payne (109) believe that the active constituent of liver extract is the anti-pernicious anemia principle. Verzár (110) on the other hand maintains that sprue is an adrenocortical insufficiency rather than a dietary deficiency and that liver extract is potent because it provides flavin phosphoric acid, which he claims the body is unable to synthesize in the absence of the adrenocortical hormone.

That various monosaccharides are absorbed at different rates has been shown by Westenbrink & Gratama (111) to be true not only for the rat, but for pigeons and frogs as well. Verzár & Wirz (112) have continued their studies on the factors affecting selective absorption of sugars in rats. They report that the rate of absorption of glucose is thirty per cent faster in the upper part of the small intestine than in the lower. Xylose, which is not selectively absorbed, is absorbed at the same rate along the entire small intestine. Since it was found that cooling the animals to 24°C decreased the absorption of glucose, particularly in the upper intes-

tine, without affecting the absorption of xylose, it was concluded that selective absorption is specifically inhibited at lower temperatures. In favor of this view was the finding that the rate of absorption of glucose in the cooled animal was no longer independent of the glucose concentration in the intestine. Since iodoacetic acid inhibited absorption mainly in the upper intestine and its effect was decreased by cooling the animals, it was concluded that iodoacetic acid also interferes with selective absorption. Laszt & Verzár (113) also claim that glucose and xylose are absorbed at practically the same rates in well-developed adrenal insufficiency, indicating that adrenalectomy interferes with selective absorption. Judovitz & Verzár (114) further report that although the absorption of glucose and galactose is markedly reduced after adrenalectomy, that of xylose, sorbose, mannose, and arabinose is not affected. Furthermore, the differential absorption rates for upper and lower segments of the small intestine are absent after adrenalectomy. Deuel, Hallman, Murray & Samuels (115), who used adrenalectomized rats maintained in good health by the administration of salt solution, were unable to detect any disturbance of glucose absorption. Apparently, therefore, the cortical hormone is not necessary for selective absorption.

In regard to the absorption of amino acids, Doty & Eaton (116) report that histidine is more rapidly absorbed than lysine, and (117) that, whereas the rates of absorption of lysine, arginine, and histidine increase with the quantities administered, the rate of absorption of glycine is independent of the amount administered. Höber & Höber (118) claim that amino acids, like certain sugars, are selectively absorbed since their absorption rates are greater than would be anticipated on the basis of their molecular volumes, and since the percentage absorption decreases with the concentration in the intestinal lumen. As a result of their investigation of the absorption of polyhydric alcohols, aliphatic amines, and fat-soluble compounds, Höber & Höber conclude that molecular volume, fat solubility, and chemical affinities for the mucosal cell membranes are factors which regulate the rate of absorption of those substances which are not selectively absorbed.

Laskowski (119) has shown that phosphates are absorbed by diffusion, and that organic phosphates are absorbed at rates which depend upon the rapidity with which they are hydrolyzed in the

intestine; this suggests that organic phosphates are not absorbed as such. Cohn & Greenberg (120) report that radioactive "labeled" phosphorus is absorbed most rapidly during the first two hours, but that thirty to forty per cent remains unabsorbed. Of the absorbed phosphorus twenty to thirty per cent appeared in the urine in eight hours, and three per cent was excreted by way of the large intestine.

Ingraham & Visscher (121) have continued their studies on the ability of the absorbing intestinal epithelium to perform osmotic work. They have shown that when a mixture of isotonic sodium chloride and sodium sulphate is placed in the intestine of dogs, the non-absorbable sulphate remains in the intestine, retaining with it an equivalent amount of sodium and sufficient water to maintain isotonicity. The chloride ion, on the other hand, is almost completely absorbed, in spite of the fact that the concentration of chloride in the intestine is only one two-hundredth of that of the blood into which it is "diffusing". The reverse situation has also been demonstrated; when sodium chloride and magnesium chloride are placed in the intestine, the magnesium ion remains behind while the sodium is absorbed against a high concentration gradient. In the case of sodium chloride and magnesium sulphate, the former salt is absorbed without the latter. Absorption of this type cannot be explained on the basis of simple osmosis and diffusion, because the concentration gradients are in the wrong direction, and because the non-diffusible ions remaining in the lumen should, according to physical processes, withdraw fluid from the blood into the intestine rather than the reverse, since the non-diffusible osmotic pressure in the intestine would be higher than that in the blood. Clearly some process requiring the expenditure of energy and the performance of osmotic work is required to accomplish this process. Ingraham & Visscher have shown that poisons such as mercury, arsenic, and cyanide prevent this process of univalent ion "impoverishment" and, in the case of cyanide, reversibly. These poisons also prevented the normal accumulation of ammonium ions in the intestinal contents, which normally may reach a level several hundred times higher than that of the blood. They conclude that the appearance of ammonia in the intestinal contents is the result of metabolic activity on the part of the functioning intestinal mucosa. That the absorbing cells may not be responsible



for the production of ammonia, however, is suggested by the well-known fact that gastric juice contains ammonia in high concentration, presumably as a result of the action of urease which has been located in the mucous neck cells by the Linderstrøm-Lang technique (122).

In order to explain the performance of osmotic work by the intestinal epithelium, Ingraham, Visscher & Peters (123, 124) have proposed a theory which they have developed in mathematical terms. They assume that pure water passes into the intestine at a constant rate, and that a solution of sodium chloride is absorbed at a constant but higher rate. The result is a constant rate of disappearance of fluid from the intestine which is clearly supported by their data. They also assume that the sodium chloride concentration of the absorbed fluid at any moment is the same as that of the intestinal contents at that moment. From these assumptions they derived an equation which described the process of absorption, and which, when satisfied with experimental data, permitted an estimation of the rate at which pure water enters the intestine. This was found to be 30 cc. or 300 cc. (typographical error?) per hour in a 20 cm. segment of intestine. It seems to the reviewers that the assumption that pure water is secreted into the intestine, and that the univalent salt is absorbed in increasingly hypotonic solution, does not provide an explanation for the ability of the intestinal mucosa to perform osmotic work, but does introduce a new difficulty — the explanation of the secretion of pure water by the intestine.

It has been suggested that water-soluble substances are absorbed by diffusion through the intercellular substance of the intestinal epithelium. The evidence described above that even absorption of sodium chloride may require active intervention on the part of the cells is contrary to this suggestion. Lampa (125) has recently investigated the problem by following histologically the absorption of vitamin C from the intestine in guinea pigs. When the animals were placed on a vitamin-C deficient diet, no vitamin could be detected in the intestinal mucosa by a silver nitrate reaction. These animals were then given ascorbic acid by mouth and killed at intervals thereafter. Histological examination of the mucosa revealed that the vitamin was not absorbed intercellularly, but intracellularly, along the Golgi apparatus. After passage through

the epithelial cells, the vitamin accumulated in the histiocytes of the tunica propria, the same cells which were found to stain with injected trypan blue.

Kokas & Ludány (126) have continued their study of the humoral control of the movements of the intestinal villi and their role in absorption. Movements of the villi were counted by means of a microscope in an exposed area of the jejunum of dogs under chloralose anesthesia. Although the introduction of hydrochloric acid into the duodenum stimulated movements of the villi, glucose under the same conditions was without effect. The increased motility of the villi following the introduction of acid into the duodenum was found to augment the absorption of glucose by approximately 20 per cent. Absorption of glucose from the colon was not influenced. The humoral nature of the mechanism was demonstrated by cross-circulation experiments involving carotid-to-carotid anastomoses. The presence of acid in the duodenum of the donor dog stimulated movements of the villi and increased the absorption of glucose by 20 per cent in the recipient dog. Neutralized extracts of the duodenal mucosa when placed in the duodenum stimulated the villi and hastened absorption, indicating that the active principle, villikinin, which is not attacked by enzymes, is readily absorbed from the intestine. The analysis of the problem of villikinin now appears to be complete.

According to Gardner & Burget (127) the presence of calcium chloride in the intestinal contents delays, and the presence of potassium chloride hastens, the absorption of glucose from intestinal segments in dogs and cats. Lajos (128) reports that both atropine and morphine delay intestinal absorption of glucose in rats. Ephedrine is without effect on glucose absorption in dogs according to Van Liere, Northrop & Sleeth (129). The same authors (130) have shown that hemorrhage to the extent of 3.2 per cent of the body weight in dogs inhibits water absorption, hastens absorption of saline solution, and has no effect on glucose absorption. Bellini & Pescetto (131) were unable to influence the rate of glucose absorption from Thiry-Vella fistulae in dogs by either raising the blood sugar by the intravenous administration of glucose, or by lowering it by injections of insulin. Strauss & Walzer (132) have used the Prausnitz-Küstner reaction in monkeys to demonstrate the absorption of unaltered protein allergens from the intestine.

*Pancreas.*—Crittenden & Ivy (133) have investigated the rôle of neural factors in pancreatic secretion in dogs. In order to eliminate completely hormonal and secretogogic influences on the pancreas, the animals were completely enterectomized in semi-chronic experiments. Under these conditions the pancreas was found to secrete continuously from 0.2 to 9.0 cc. of juice per hour. A small psychic secretion could be obtained in response to sham feeding with meat broth. Stimulation of the vagus nerve usually augmented pancreatic secretion if the nerve had been sectioned five days before in order to permit the cardiac fibers to degenerate. Contrary to expectation vagus stimulation usually reduced the response to a standard dose of secretin. Eserine and pilocarpine usually augmented secretion; acetylcholine frequently inhibited, particularly when given after eserine.

Babkin, Hebb & Sergeev (134) have investigated the effects of stimulation of the splanchnic nerves on pancreatic secretion. They report that rhythmic stimulation produces a slight flow of pancreatic juice, and that following this the histological appearance of the gland resembles that following vagus stimulation. The secretory effect of splanchnic stimulation was potentiated by removal of the adrenal gland on the side stimulated, by eserine and by nicotization of the celiac ganglion. It was inhibited by atropine and cocaine. Epinephrine alone did not stimulate secretion and histologic examination revealed no discharge of zymogen granules. From these results it was concluded that the splanchnic nerves to the pancreas are mixed nerves, containing both cholinergic and adrenergic fibers of which the former are excitatory and the latter inhibitory.

Tanturi, Ivy & Greengard (136) have shown that secretin is a true chologogue, for it stimulates the formation of bile by the liver after complete removal of the gastrointestinal tract. Its effect is not secondary to its action on the pancreas as previously believed. Agren & Hammarsten (137) have reported that their crystalline secretin may be digested by aminopolypeptidase without loss of activity. Ten amino acids are split off in the process.

Handelsman (138) has published a complete review of the rôle of the external secretion of the pancreas in digestion.

*Gall bladder.*—Schmidt & Ivy (139) have made a study of the comparative physiology of the rate of bile secretion, the resistance

of the sphincter of Oddi, and the "physiologic capacity" of the gall bladder in different species of animals. The "physiologic capacity" is numerically equal to the number of hours of bile secretion which the gall bladder is able to store and concentrate. It was observed that as the sphincter resistance increases from species to species the "physiologic capacity" also increases, and that as the rate of bile secretion decreases the concentrating power of the gall bladder increases. These results indicate that the general function of the gall bladder is to store bile for purposes of digestion, and perhaps also for regulation of pressure within the biliary passages in those cases where the sphincteric resistance is high. Gorham & Ivy (140) have made an exhaustive study of the presence or absence of the gall bladder throughout the phylogenetic series. They found that carnivorous mammals, with the exception of whales, possess a gall bladder; animals with intermittent feeding habits are most apt to retain the structure, whereas the herbivores with continuous feeding habits are most apt to lose it. No evidence was found which indicated that the sphincter of Oddi preceded the development of the gall bladder. This also suggests that the main function of the gall bladder is to store bile.

Viehover (141) has observed gall-bladder evacuation in the scaleless telescope fish which, being transparent, permits observation of the green gall bladder without artificial aids and without injury to the animal. As little as 0.03 mg. of Ivy's cholecystokinin injected intravenously produced evacuation of the gall bladder within two to two and one-half hours.

Sussman (142) reports that the gall bladder remains uncontracted for as long as five days in human subjects when fasted or maintained on a carbohydrate diet. After an egg-yolk meal the normal gall bladder decreases to less than one-half its original size within thirty minutes. Morphine prevents evacuation under these conditions, presumably because of its property of producing spasm of the sphincter of Oddi as demonstrated by Doubilet & Colp (143). Flexner, Bruger & Wright (144) report that in amyotized cats acetyl- $\beta$ -methylcholine produces contraction and benzedrine produces relaxation of the gall bladder. Many of the animals failed to survive the excessive dose of acetyl- $\beta$ -methylcholine; it would be of interest to know what effect sublethal doses might have.

Gerdes & Boyden (145) have shown that during pregnancy

the ability of the gall bladder to concentrate the dye, iso-iodoikon, is reduced. Although during the first trimester of pregnancy the rate of emptying after the Boyden meal is not altered, during the remainder of pregnancy it is markedly retarded. This is attributed to hypertonicity of the sphincter of Oddi. This biliary stasis probably accounts for the high incidence of gall stones in women who have borne children.

*The colon.*—Wright & Florey (146) have investigated the effects of the autonomic nerves on the secretory activity of the colon in decerebrate cats. They report that stimulation of the nervi erigentes stimulates secretion by the colon. Stimulation of the hypogastric nerves alone had no effect, but when stimulated simultaneously with the nervi erigentes the effect of the latter was inhibited. The effect of stimulating the parasympathetic nerves was potentiated by eserine and abolished by atropine; acetylcholine and pilocarpine possessed secretory activity. They observed that the resting mucosa could apparently completely reabsorb the secretion stimulated by any of the above means. The only enzyme that could be detected in the secretion was dipeptidase.

Masuda (147) has reported the effects on colon motility which follow stimulation of the autonomic nerves in cats, dogs, rabbits, and guinea pigs. The movements were observed either after the intestines had been spread out in a saline bath, or when recorded cinematographically through an abdominal window. In all animals they found that vagus stimulation produced movements in the cecum, proximal colon, and ileocecal sphincter. Stimulation of the sympathetics inhibited the motility of the cecum and proximal colon, and slightly relaxed the ileocecal sphincter. Stimulation of the pelvic nerves in cats and dogs produced shortening movements in the distal colon, whereas in rabbits peristaltic waves followed one another analwards in the distal colon.

Puestow (148) has studied the effect of drugs on colon and intestinal motility in a patient who had been operated upon in such a way as to permit direct observation of intestinal movements. At no time were the small intestine and colon manifesting motility at the same time. He claims that morphine, paregoric, eserine, acetylcholine, and prostigmin stimulate intestinal motility and paralyze the right colon, whereas pituitrin activates the right colon and inhibits intestinal movements. Galapeaux, Templeton &

Borkon (149) report that the introduction of gall-bladder bile into the rectum of dogs markedly depresses colon motility as recorded by three tandem balloons, while producing defecation with tenesmus. Galapeaux & Templeton (150) also report that filling a dog's stomach with one-half pound of yeast and 1100 cc. of buttermilk augments colon motility and produces defecation in untrained animals. In trained animals the defecation is deferred until they are put in a suitable environment. Watkins (151) has shown that dilatation of the rectum in urethanized cats lowers the blood pressure and relaxes the nictitating membrane, whereas dilatation of the anal sphincter produces the reverse effects.

*Intestinal flora.*—Weinstein & Weiss (152, 153) have shown that the character of the intestinal flora of rats may be easily altered by including certain fruits in the diet. The animals were first placed on a meat diet until *L. acidophilus* had disappeared from the feces. The subsequent addition of banana powder, apple powder, cranberry powder or raisins soon restored *L. acidophilus* to the feces. Tomatoes, prunes, charcoal and kaolin were unable to accomplish this. Weinstein, Weiss & Gillespie (154) have also investigated the rôle of intestinal pH in regulating the intestinal flora, using the glass electrode for the determination of pH. The rats were maintained on various diets in order to provide a wide range of bacterial types. In the small intestine no correlation was found between the pH and the percentage of *L. acidophilus* in the intestinal flora. In the colon however, when a large number of determinations were averaged a correlation became apparent. The fact that the correlation became apparent only when averages were used suggests that some factor besides pH is of considerable importance. Robertson (155) has shown that young rats maintained on a calcium-free diet develop stasis and dilatation of the cecum and colon, and excrete ingested barium sulphate more slowly than controls. This condition (156) was accompanied by an increase in the acidity of the cecal and colonic contents. Bacterial counts and dilution tests revealed the presence of twice as many microorganisms per unit weight in the contents of the colon and cecum of the deficient animals as in controls. Eppright, Valley & Smith (157) have shown that rats maintained on a salt-free diet rapidly lose their normally high *L. acidophilus* count in their feces. The addition of Osborne's salt mixture to the diet rapidly restored the nor-

mal count. It was then shown that calcium and phosphorus together, but neither alone, were effective in restoring *L. acidophilus* to the feces. Sokolov (158) reports that "trilactic", a polymolecular form of lactic acid which slowly liberates the monomolecular form in the intestine, increases the *L. acidophilus* count of feces and that this effect is augmented by including lactose and calcium and phosphorus. The mechanism of action of these substances is not clear, but it now appears that the bacterial flora can be controlled successfully by dietary measures, without inoculation with the desired microorganism.



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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY  
NORTHWESTERN UNIVERSITY MEDICAL SCHOOL  
CHICAGO, ILLINOIS

## PHYSIOLOGY OF THE LIVER\*

BY FRANK C. MANN AND J. L. BOLLMAN

*Division of Experimental Medicine  
The Mayo Foundation, Rochester, Minnesota*

Several very satisfactory review articles concerning the physiology of the liver were published in 1938. Among these should be mentioned the reviews of Ivy & Crandall and Lundsgaard. Long & White in their article on intermediary carbohydrate metabolism have reviewed much of the literature pertaining to the liver in the field of carbohydrate metabolism. Reviews which cover the field of applied physiology of the liver include those by Crandall & Ivy; Ravdin; Rhoads, Frazier & Ulin; Boyce & McFetridge; and Sweet.

### HEPATIC CIRCULATION

McMichael found that in the cat the liver obtains about two-thirds of its oxygen from the portal vein under normal conditions of blood pressure. Obstruction of the portal vein to a lobe causes central degeneration in the lobules. He found also that when the blood pressure is lowered by hemorrhage and shock, the oxygen content of the portal venous blood is proportionately diminished and the liver becomes more and more dependent on the hepatic artery for its oxygen. In the rabbit the liver is almost entirely dependent on the hepatic artery for its oxygen and gets only an insignificant amount from the portal blood. Irritation of the hepatic nerves by a ligature may cause a temporary diminution of the flow through the liver by causing vasoconstriction of the portal venules within the liver. This effect, however, is so transient that it does not play any significant part in determining the liver degeneration which results from ligature of the hepatic artery. Temporary occlusion of the hepatic artery does not lead to reactive hyperemia in the liver.

Cherry & Crandall studied the hepatic circulation time in unanesthetized dogs by observing the time required for potassium cyanide injected into the hepatic and later into the portal veins to reach the carotid sinus. This was determined by the onset of increased respiratory excursions as recorded with a pneumograph.

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The difference in time required represented the hepatic circulation time. In five normal dogs the hepatic circulation time was determined as thirteen seconds. Denervation of the adrenals, pancreatectomy or hypophysectomy had no significant effect on the hepatic circulation time.

#### BILE AND BILIARY SYSTEM

Tanturi & Ivy (1) studied the effect of vascular changes in the liver and the excitation of its nerve supply on the formation of bile. They found that the formation of bile is definitely influenced by the intrahepatic vascular pressure. An acute increase in portal and hepatic venous or hepatic arterial pressure decreases bile formation. Occlusion of the hepatic artery augments bile volume output at least for a few hours. This might be expected on the basis of the relatively low secretory pressure of bile and the exposure of the bile capillaries to the effects of changes in vascular pressure. The authors found also that stimulation of the sympathetic nerve supply of the liver decreases bile formation; the evidence indicates that the decrease is due to vascular or mechanical changes in the liver rather than to true inhibitory secretory nerves. Tanturi & Ivy tentatively concluded that an increase in blood flow through the liver augments bile output in acute experiments, except that when the increase in blood flow is associated with an increase in intrahepatic vascular pressure.

These same authors (2) also studied the existence of secretory nerves in the vagi for reflex excitation and inhibition of bile secretion. They found that a definite excitatory-secretory effect on bile formation may be obtained in the dog and the monkey, but not in the cat or the rabbit, by stimulating the peripheral end of the vagus nerve in the neck five days after it has been sectioned. The secretory effect is not accompanied by a change in systemic blood pressure. During the period of stimulation an inhibitory-secretory effect may be observed in the dog, the monkey and the cat. Prolonged stimulation of the intact vagus nerve in the neck in the dog, but not in the cat or the rabbit, results in prolonged augmentation of bile formation after the heart has escaped from vagus inhibition. Augmentation of bile secretion may be obtained in the dog if the central end of one vagus is adequately stimulated when the other vagus is intact. Section of the second vagus abolishes the augmentative effect of stimulation of the central end of the



other vagus. This demonstrates the existence of a reflex vagal augmentation of bile formation in the dog. With the vagi sectioned, the stimulation of the central end of one reflexly diminishes bile formation through paths in the spinal cord. Pilocarpine and prostigmine in the dog first inhibit and then definitely augment the formation of bile. The excitatory-secretory effects were obtained in dogs devoid of the gastro-intestinal tract, spleen and pancreas. Tanturi & Ivy found that motor nerve fibers which exert an inhibitory and an excitatory effect on bile formation by the liver exist in the vagi of the dog. The liver is quite analogous to the pancreas in regard to secretory innervation. Sensory fibers, which reflexly excite bile formation by way of the vagus nerves and which reflexly depress bile formation (hepatic vasoconstriction) by way of the spinal cord, are also present in the vagi.

Kocour & Ivy devised a new method for determining the volume output of bile in the dog under different experimental conditions by the use of continuous suction to the tube in the common bile duct. Under controlled conditions the volume output of the secretion was extremely constant. The bile output of seven dogs on a regimen of no food and without the return of bile ranged from 6 to 10 c.c. per kg. per day. On a mixed diet fed every six hours without the return of bile to the intestine, the output ranged from 13 to 18 c.c. per kg. On the same diet with the return of a standard amount of bile, it ranged from 24 to 27 c.c. per kg. per day. Hot weather had no effect on volume output when the appetite was normal. Water, as it is usually ingested, had no effect on bile volume output.

When a fasted animal not receiving bile was fed a mixed diet or meat, the bile output increased during the first and second hours after eating and continued at the higher rate during subsequent six-hour feedings. If bile was returned with the first and subsequent six-hour feedings, a typical secretory curve was obtained after each feeding, the largest volume being secreted usually during the second hour. On maintaining the caloric intake of a mixed diet constant, the quantity of bile secreted on a six-hour feeding schedule was somewhat larger than on a twenty-four hour single feeding schedule. The maximal output from a single meal occurred during the first six hours, but the effect of the meal was evident for twelve hours. No difference between the day and night output of bile existed if the dog was fed a mixed diet every six hours dur-

ing the twenty-four; but if a meal was not fed at midnight the night secretion was less than that of the day. Feeding meat more frequently than every six hours but maintaining the caloric intake constant did not augment bile output.

The potency of meat as a stimulant of volume output of bile, which is quite generally agreed to in the literature, was confirmed by the authors. The addition of meat to a mixed diet also increased bile output. One hundred grams of beef liver when added to a mixed diet produced from eighteen to twenty per cent more bile than did beef muscle or beef heart. Per gram weight, hepatic bile is considerably more potent than any food and gall-bladder bile is more potent than hepatic bile.

The authors found that the ingestion of 20 c.c. of olive oil by a fasting dog, with or without the return of bile to the intestine, stimulated bile output in most, but not all, dogs to the same extent as 125 gm. of raw, ground meat. The stimulating effect of olive oil (fed alone) disappeared when it was repeatedly ingested at six-hour intervals; the disappearance of its stimulating effect occurred sooner when bile was not returned to the intestine. When 60 c.c. of olive oil were added to a mixed diet, bile output was not stimulated. Thus, olive oil is not a constantly reliable excitant of bile secretion, except when fed once or twice after a period of fasting.

When *d*-glucose (35 gm.) was given orally or intravenously without bile being returned to the intestine, bile output was decreased from 20 to 35 per cent and the depression of bile output might last for from six to eighteen hours. When *d*-glucose was injected intravenously and bile was being absorbed from the intestine, bile output was increased. However, after a third injection, made at six-hour intervals, bile secretion might be inhibited. When *d*-glucose (70 gm.) was added to a mixed diet, bile being returned, an unpredictable slight increase or decrease occurred. Meat and liver were the only foods studied which, when added to a mixed diet, consistently increased volume output of bile. Vomiting, nausea or anorexia decreases bile output. In the presence of a definite hepatitis, dehydrocholic acid administered intravenously or bile introduced into the intestine had no or very little effect on bile volume output.

Höber & Moore perfused the isolated livers of frogs and rabbits with Ringer's solution containing dyes and various other substan-

ces. They found that secretion is stimulated by bile acids, higher fat acids, carbamates and saponin. They believed that the dispersion of the cell colloids could be considered a controlling factor in the secretory activity of the liver.

Doubilet & Colp studied the total bile-acid to cholesterol ratio in human and canine bile and found that in human bile it was about 20; in early chronic cholecystitis the ratio averaged 13, while in advanced cases it was 7.7. This increase in cholesterol increased the danger of precipitation only slightly, since the relative amount of the most effective solvent, desoxycholic acid, is greatly increased. In acute cholecystitis, the ratio was 6. Here the high cholesterol concentration increases its precipitation. If the liver is injured, as in inflammation or acute obstruction, the excretion of cholesterol is much more markedly reduced than is the excretion of bile acids. It is probable that excretion of biliary cholesterol is controlled by some independent function of the hepatic cells, which is related to the lipide metabolism. The bile acid-cholesterol ratio is readily raised by the oral administration of large amounts of bile salts, especially cholate. This may reduce the danger of precipitation.

Beck, Krantz, Feldman & Carr studied the hydrogen-ion concentration of the gallbladder bile of the dog and found that in the normal dog and in the dog after feeding of ox bile the hydrogenion concentration increased with the concentration of the bile.

Feldman, Morrison, Carr & Krantz found that human gallstones inserted into gallbladders of dogs did not dissolve but often increased in weight when the bile was alkaline. The authors believe that a change in pH of gallbladder bile is an important factor in gallstone formation.

Kohlstaedt & Helmer made post-operative determinations on human fistula bile in five cases in which bile salts had been administered orally and in five control cases in which no bile salts were given. Desoxycholic and taurocholic acids, total conjugated bile acids and cholesterol were determined. The composition of human fistula bile was constant despite a variable diet. The oral administration of bile salts brought about a marked increase in the concentration of bile acids in the bile but did not increase the cholesterol content. The proportion of bile acids conjugated with taurine was not increased to the same level as the other bile acids. The volume

of bile did not parallel the rate of excretion of either bile acids or cholesterol.

Correll, Berg & Cowan found that kynurenic acid was excreted in the bile after tryptophan administration in dogs. *dl*-tryptophan produced much less kynurenic acid than *l*-tryptophan probably because *d*-tryptophan does not undergo direct conversion to kynurenic acid in the dog.

Galapeaux, Templeton & Borkon found that the injection of 10 to 20 c.c. of dog's gallbladder bile into the colon produced a marked depression in colonic activity, which lasted from fifty to one hundred minutes.

Atkinson & Ivy studied the effect of the removal of a normal gallbladder on the metabolism of lipides and concluded that the rise in fasting plasma lipide levels obtained after cholecystectomy is not specifically due to removal of the gallbladder; it may also be obtained after liver damage or tissue injury and for a short time after ether or chloroform anesthesia. By studying the blood lipides (total fatty acids, free and total cholesterol) before and after cholecystectomy both under the condition of fasting and during the digestion and absorption of fat, they could not demonstrate that the gallbladder possesses a function of regulating the metabolism of lipides.

Flexner, Bruger & Wright studied the effects of the autonomic drugs on the biliary system and found that the subcutaneous administration of acetyl- $\beta$ -methylcholine chloride (mecholy) to cats is followed by contraction of the gallbladder. Benzylmethylcarbinamine sulfate (benzedrine sulfate) when similarly administered caused relaxation of the gallbladder.

#### CARBOHYDRATE METABOLISM

Bachmann, Haldi, Wynn & Ensor and Haldi, Bachmann, Ensor & Wynn observed the effects of high *d*-glucose and high fructose diets on the body weight and on the fat, glycogen and nitrogen content of the liver and body. They fed to two groups of rats for ten weeks equicaloric diets of the same composition except that one contained 68 per cent *d*-glucose and the other the same percentage of fructose. The activity of the animals was limited by the restricted size of the cages in which they were kept. The average increase of weight on the two diets was the same. The ratio of

food intake to gain in weight was therefore the same for both sugars but was considerably higher for the females than for the males. The average total percentage content of glycogen and nitrogen in the entire body was the same in the *d*-glucose and fructose-fed animals. The fat content was significantly greater in the rats on the *d*-glucose diet, and the hydration of the tissues was apparently greater in the fructose-fed animals. The liver of the animals on the fructose diet was heavier in every experiment, the average weight for the group being 22 per cent greater than in the *d*-glucose fed animals. The authors attribute this hypertrophy of the liver to an increase in the work of that organ in the metabolism of fructose. The nitrogen and glycogen content of the liver was essentially the same with both diets. The percentage of fat content was greater in the liver of the *d*-glucose-fed animals but owing to the increase in weight of the liver of the fructose-fed animals the total amount of fat in the liver was the same on both diets.

These same authors allowed three groups of animals to exercise when fed on diets of the same composition except that one contained 68 per cent *d*-glucose, one the same percentage of fructose, and the other a mixture of equal parts of the two sugars. The animals were permitted free access to an exercising wheel. There were no significant differences in the gain in weight on the three diets. Spontaneous activity was greatest on the *d*-glucose ration, least on the fructose, and intermediate on the mixture of the two sugars. There was but a small difference in the body weight of the rats on these diets but the fat content of the body was considerably less than in the corresponding non-exercising experiments. The differences were accounted for by the activity of the exercising animals and it is concluded that the energy required for the exercise was supplied for the most part at the expense of body weight. The percentage of nitrogen and glycogen of the body was the same with the two sugars but the liver was appreciably larger on the fructose diet than on the *d*-glucose diet. The percentage of nitrogen in the liver was the same on all three diets and the percentage of glycogen was the same when the two sugars were fed separately but was definitely higher when the mixture of sugars was fed. The percentage of fat was higher on the *d*-glucose than on the fructose diet.

Mirski, Rosenbaum, Stein & Wertheimer found that the high glycogen reserves, especially in the liver (in their cases about four

per cent glycogen), appearing when rats are fed a diet rich in carbohydrate, are brought easily almost to vanishing point by increased demands of various kinds on the carbohydrate metabolism. The far smaller glycogen content in the case of excessive protein-feeding (1.4 per cent glycogen in the liver) is little affected by the same forms of demand. After twenty-four or forty-eight hours' fast, where there has been previous excess of carbohydrate in the diet, only vestiges of glycogen are found, but in the protein-fed animals in all the experiments taken together, there is around 1.2 per cent. With twenty-four hours' fast and simultaneous administration of phlorhizin, it is found that although the sugar excretion in the urine is usually greater than the amount of the total carbohydrate reserves, with the protein-fed animals amounts of 0.5 per cent glycogen can still be found in the liver, while with the carbohydrate-fed animals usually only minimal amounts are present. After an average of five hours' action of cold at a mean temperature of 10° C., the glycogen almost vanishes in the carbohydrate-fed animals, whereas in the protein-fed animals a mean of one per cent glycogen is found.

After three days' fasting, newly formed glycogen (on the average 1.2 per cent) is found in the liver of the carbohydrate-fed animals and a like amount in the protein-fed animals. In the fasting experiments muscle glycogen and blood sugar show differences qualitatively similar to those for the liver glycogen but quantitatively much smaller. After swimming experiments the glycogen disappears to a great extent in both series of animals. After a subsequent rest pause of four to twenty-four hours without food, the glycogen in the liver decreases still further in the carbohydrate-fed animals but in the protein-fed animals a pronounced fresh building up of glycogen in the liver is observed. Similar results were observed after injecting minimal amounts of dead bacteria (*B. paratyphosum* B).

In the animals fed with an excess of fat and with the usual protein content of the diet, a maintenance of the glycogen reserves was not observed. The actions described must therefore be connected with the disintegration of protein ("protein effect"). After removal of the adrenal glands, the protein effect no longer supervenes. It is concluded that an intensified glyconeogenesis is the most important cause of the phenomena described. Excessive catabolism of protein leads to a powerful incitement to glyconeogenesis.

genesis, so that even in starvation and in heavy claims on the carbohydrate metabolism glycogen is freshly formed in the liver.

Deuel, *et al.*, found that in rats having access at all times to ample amounts of stock diet, there was a diurnal variation in the liver glycogen from a maximum in males of 4.74 per cent at 4 a.m. to a minimum value of 1.88 per cent at 4 p.m. and in females of 4.59 to 1.15 per cent (the minimum occurring at 8 p.m.). Evidence seemed to indicate that the variations corresponded to diurnal differences in food intake. In rats fasted two days and then given a *d*-glucose test meal, the level of liver glycogen twelve hours after the test meal was remarkably constant at four-hour intervals studied during a twenty-four hour period. The results seem to indicate that the diurnal variation in the liver glycogen is entirely of dietary origin. Attention is directed to the fact that sex is a factor which must be considered in such tests. The ratio of the mean difference to the probable error of the mean difference (10.93) for the grand average shows that the level of 3.43 per cent for glycogen in the males is significantly different from that of 2.96 per cent for the females.

Seckel & Kato made chemical and histologic analyses in 168 young growing rats from one to twenty-one days old with a view to quantitative estimation of hepatic glycogen and microchemical demonstration of bile acids in hepatic cells at hourly intervals during the twenty-four hour period. Hepatic glycogen and bile acids demonstrate a reciprocal relationship in that when the one is present abundantly in the liver cells the other is proportionately reduced in amount. In rats up to the age of thirteen days both substances are more or less uniform in quantity during the twenty-four hour period, the values for hepatic glycogen being persistently low and those for bile acids relatively high. At that age, no definite diurnal cycle of liver function is demonstrable. In rats from fourteen to twenty-one days of age, however, liver function shows a definite diurnal cycle in glycogen storage and bile acid secretion. The glycogen content (reaching a much higher level than during the first two weeks of life) has its maximum during the early morning and most of the forenoon, while the minimum occurs in the late afternoon. The curve of bile acids is a mirror image of that of glycogen. The results obtained by the present experiments add further proof for the existence of a diurnal cycle in liver function paralleling similar variations in body temperature



and other metabolic functions as demonstrated by previous investigators.

Sjögren, Nordenskjöld, Holmgren & Möllerström studied the glycogen, phosphorus and calcium content of the liver of white rabbits which had been killed at various times within a period of twenty-four hours. Glycogen was also determined in the liver of animals which were maintained on a restricted diet for various periods of time. The sexes were kept separate. The liver glycogen determination in animals on an adequate diet showed a rhythmic twenty-four hour variation with two maxima. The smaller maximum appeared somewhere around 5 a.m. and the greater maximum somewhere around 3 p.m. Of the two minima, one was about 9 a.m., the other extended over a broader period around midnight. The maximum amount of glycogen seemed to bear no relation to the time of feeding in these experiments. The authors believe that the smaller peak of the maximum glycogen is alimentary in origin, while the larger peak is endogenous in origin. The liver phosphorus and calcium showed no twenty-four hour variation in animals that were maintained on an inadequate diet.

Maddock & Svedberg found that after total hepatectomy in the monkey, the typical features of the blood chemistry picture are as follows: The blood sugar falls. The urea remains constant in nephrectomized or anuric animals, and falls in animals which excrete urine. Uric acid, amino-acid and creatinine increase. There is decreased urinary secretion, decrease in total nitrogen and urea and an increase in amino-acid nitrogen, uric acid and ammonia nitrogen of the urine. Essentially the same findings were observed after total removal of the liver in the rabbit by Svedberg, Maddock & Drury.

Bergman & Drury found that the rate of utilization of *d*-glucose by the eviscerated rabbit is affected by feeding and fasting prior to operation. The tissues of the fed animals utilize *d*-glucose at a rate double that of the fasted. This increase cannot be due to insulin.

Himsworth developed a new method for hepatectomy in rabbits, which provides ligation of the blood vessels to and from the liver without anesthesia. The untreated animals died about five hours after liver removal with a terminal *d*-glucose value of about 20 mg. per cent. The decline in blood sugar appeared to be in wave-like decreases interrupted by occasional increases. Hims-

worth & Scott found that the effect of injecting epinephrine intravenously into unanesthetized rabbits from whose circulation the liver had been excluded was, in the doses employed, to accelerate the rate of removal of *d*-glucose from the blood by the peripheral tissues. The authors suggested that the accelerated removal of the *d*-glucose of the blood results from increased utilization of this sugar by the tissues under the influence of epinephrine. They presented further evidence which suggested that there exists in the extrahepatic tissues a store of carbohydrate which can supply glucose to the blood. The waves which interrupt the spontaneous fall of the *d*-glucose of the blood after exclusion of the liver from the circulation may be attributable to the peripheral effect of epinephrine secreted in response to the falling *d*-glucose of the blood. The changes in the curve of the blood sugar after the injection of epinephrine appear to give a decrease in the amount of sugar in the blood followed by an increase to about the same level as might be expected without the epinephrine.

Griffith, Lockwood & Emery found that epinephrine injected into eviscerated cats did not materially increase the blood lactic acid and that the muscles in the absence of the liver do not liberate lactic acid under the influence of epinephrine. Petiteau observed a rapid rise in blood lactic acid shortly after hepatectomy in the dog. Hepatectomy performed several days after pancreatectomy produced a much greater rise in the blood lactic acid, and the author believes that the removal of the liver suppresses the transformation of lactic acid to *d*-glucose.

Ussing used deuterium in studying the formation of glycogen in the liver. When glycogen was suspended in water that contained deuterium, three out of ten hydrogen atoms of glycogen were freely exchanged with deuterium. When glycogen was formed in the liver of rats fed *d*-glucose and deuterium, the liver glycogen content of deuterium was twice the estimated amount of deuterium. From these values it would appear that a new configuration of the hydrogen atoms of the monoglucides occurs when glycogen is formed in the liver. Althausen found that less deposition of glycogen occurred in animals with extremely damaged livers when *d*-glucose was administered by a stomach tube or intravenously.

Kosterlitz found that when rabbits were fed galactose, galactose phosphate was present in the liver a few hours after feeding. Imanaga found that liver slices in oxygenated Ringer's solution

formed *d*-glucose from dihydroxyacetone but did not do so in the absence of oxygen or when excessively fatty livers were used. Glyceraldehyde formed only about one-half as much sugar and was not influenced by the presence or absence of oxygen. Snyder & Johnson could not find evidence of an increased output of sugar or potassium during perfusion of turtle liver following stimulation of the vagus or the administration of acetyl- $\beta$ -methyl choline. Vendég found that the administration of insulin to dogs whose livers had become extremely fatty after a prolonged fat diet produced little change in the glycogen or fat content of the liver or in the fat or acetone of the blood. When insulin and glucose were given there was an increase in the liver glycogen and a decrease in liver fat as well as a disappearance of acetone bodies from the blood. The administration of sodium butyrate increased both the liver and muscle glycogen and caused a marked increase in the blood fat and acetone bodies. Vendég expressed the belief that these experiments indicate that acetone bodies are formed when carbohydrate is being formed from fatty acids.

#### FAT METABOLISM AND FATTY DEGENERATION

Yannet & Darrow (1, 2) using cats varying in age from four weeks to about six months, found that the livers of the older animals showed an increase in the concentration of lipid phosphorus and nitrogen and a decreased concentration of sodium, chlorides and water. These changes are all apparently associated with the deposition of lipid and are related to the nutritional status of the animals rather than to growth. Except for a decrease in the intracellular concentration of potassium of the livers of the older animals, no changes were demonstrated in the concentration of nonlipid nitrogen and phosphorus or potassium in the intracellular fluid of the tissues studied. They also found that in hyperthermia in cats there was a shift of water from the cells of the brain to the extracellular fluid accompanied by no loss of potassium, nitrogen or phosphorus. While these changes were present in the brain, no significant changes in the water or electrolyte content of the liver and muscle could be found.

After repeated administration of carbon tetrachloride to dogs, Winter observed a decrease in the fatty acids of the blood as well as a definite decrease in the iodine number of the blood fat. The fat stores in the body appeared to be depleted, particularly un-

saturated fatty acids, probably as a result of interference with the function of the liver in the desaturation of the fat. Neale & Winter found that sodium xanthine protected the liver of rats against liver damage due to chloroform or carbon tetrachloride.

Menon (2), using rats, guinea-pigs and rabbits, produced cirrhosis of the liver by long continued administration of manganese and senecionine. He found that during the development of hepatic cirrhosis definite fibrotic and proliferative reactions, which appeared to be independent of portal stasis, occurred in the spleen. In an earlier paper (1) he had reported finding that experimental portal congestion did not produce comparable changes in the spleen.

Chaikoff, Connor & Biskind found that depancreatized dogs maintained with insulin developed fatty livers, which later became cirrhotic at the time the fat content of the liver was usually found to return to normal. Connor observed similar cirrhotic changes in two persons suffering from diabetes and in a study of alcoholic intoxication concluded that cirrhosis may develop after fatty infiltration of the liver.

Bogdanovitch & Man injected theelin, antuitrin-S and other substances into guinea-pigs and found that the females treated with theelin showed the most marked increase in fatty acid content of the liver. Injections of antuitrin-S caused an increase in the fat content of the liver, blood and muscle in both males and females. Chevillard, Hamon & Mayer observed that the accumulation of fat in the liver of fasting animals occurred earlier in the mouse than in the rat or guinea-pig, and later in the rabbit. MacKay & Carne found that the liver during twenty-four hours of fasting after partial hepatectomy becomes very fatty. Higher fat levels were found in the female than in the male rats. The deposition of fat was inhibited by previous adrenalectomy but was not influenced by administration of choline.

Sveinsson fed rabbits a diet containing twenty to forty per cent cocoa fat and found that the livers contained as much as thirty to forty per cent fatty acids. Administration of insulin or epinephrine appeared further to increase the fat content and decrease the glycogen content of these livers. Halliday found that fatty livers developed in vitamin-B<sub>6</sub>-deficient rats and that the addition of choline brought about some reduction in the fat content of the liver. Rubin, Present & Ralli could not find an altera-

tion from normal in the fat content of the liver of dogs receiving various types of fat with and without the addition of lecithin.

Loizides studied the effect of cholesterol feeding on lipid deposition in the liver of rats and found that on a diet causing "fat" fatty livers in rats, the degree of glyceride infiltration was proportional to the amount of fat in the diet. There was a small increase in the amount of cholesteryl esters, which does not appear to be related to the percentage of fat in the diet. On a diet causing the "cholesterol" fatty liver, successive increases in the percentage of fat in the diet caused progressive and large increases in the cholesteryl ester content of the liver. Considerable increases in the amounts of glyceride also occurred, and the "cholesterol" fatty liver always contained much more glyceride than the "fat" fatty liver at any given level of dietary fat. Small increases in the amounts of free cholesterol also occur with increases in the amount of dietary fats, and these become greater when cholesterol is present in the diets.

Maclean, Ridout & Best found that the presence of choline in the diet favored a normal distribution of fat between the liver and the body depots and prevented failure of certain functions of the liver which are interfered with by excessive fat deposition. Mukerji & Guha found that fatty infiltration of the liver could be produced in rabbits by a large injection of anterior pituitary extract and that choline was ineffective in preventing this change. Best & Campbell produced an increase in the liver fat by injection of anterior pituitary extract. The increase was greater in guinea-pigs and mice than in rats. The female guinea-pigs showed a greater increase than the males, and ketogenesis was greater in the females than in the males. Foglia & Mazzocco injected an alkaline extract of anterior pituitary into dogs and found a large increase in the liver fat as well as an increase in liver glycogen.

Channon, Loach *et al.* observed the effects of proteins in the prevention of dietary fatty livers by feeding various proteins both as a sole dietary protein and as supplements to the basal protein ration. All the proteins investigated had some lipotropic action. From the results obtained it seems a strong possibility that the lipotropic activity of proteins is due to some constituent amino-acids.

Channon and his collaborators found homocholine to have a greater effect in controlling the percentage of fat in the livers of

rats fed on diets which caused either fat or cholesterol fatty livers. Tripropyl- $\beta$ -hydroxyethylammonium hydroxide had no effect. Triethyl- $\beta$ -hydroxyethylammonium hydroxide had about as much effect as choline. They found that triethyl- $\beta$ -hydroxyethylammonium hydroxide was not detectable in the livers of animals even after the fat content had returned to normal. No definite evidence was therefore obtained, but the choline compounds act by way of lecithin formation. Methionine is about one-twelfth as effective as choline in preventing glyceride deposition in the liver and the substance also decreases the deposition of cholesterol in the liver of animals receiving the cholesterol diets. Cystine greatly increases the deposition of fat and appears to be antagonistic to methionine. Somewhat similar observations were reported by Tucker & Eckstein.

Mann, Woodward & Quastel and Bernheim & Bernheim made interesting studies of the oxidation of choline by choline oxidase in the liver. MacKay & Barnes (1, 2) and Aylward & Holt believe that the reduction in the liver fat caused by the pancreatic extract lipocaic is due to its choline content and to the protein which it contains. Shapiro & Wertheimer found lipocaic effective in removing excessive liver fat in the rat without producing any definite alteration in acetone excretion or changing the amount of fat or sugar of the blood.

Ralli, Rubin & Present found fatty changes in the liver of dogs thirteen to fifteen weeks after ligation of the pancreatic ducts. Inclusion of a lecithin supplement in the diet did not prevent the formation of a fatty liver and the authors were unable to demonstrate the fat metabolizing hormone of the pancreas. Best & Ridout found that the lipotropic effect of a pancreatic extract was similar to that exerted by an equal amount of dietary casein. Channon, Loach & Tristram found that extracts of the pancreas prevent fat deposition in the liver to a greater extent than can be attributed to their content of choline. Not more than one-third of the activity could be ascribed to this substance and the protein content was insufficient to account for the remainder of the action, so that it would appear that some other substance in the pancreas is involved in the prevention of fat deposition in the liver.

Hahn & Hevesy (1) found that the distribution of radioactive phosphorus in egg yolk and in the liver and blood of chickens indicated that the liver is responsible for the formation of phos-

phatides. They also found that phosphatides were produced when the liver was perfused with a radioactive sodium phosphate.

Lorenz, Chaikoff & Entenman found a pronounced increase in the neutral fat in the liver of the female bird at the onset of maturity without any change in the phospholipide or cholesterol ester content. The amount of liver cholesterol or phospholipide did not appear to be correlated with any of the measures of ovarian activity. The total fatty acids of the liver gave some indication as to the rate of production of eggs. Artom, Sarzana & Segré used radioactive phosphorus to study the phospholipide distribution in rats. The distribution of radioactive phosphorus in the tissues indicated that the liver and the intestine were specifically active in the formation of phospholipides. The formation of phospholipides seems to be increased in the animals fed a fat diet to a greater extent than in animals receiving carbohydrate diets.

Barrett, Best & Ridout (1, 2) used deuterium to determine the source of the excess fat which appears in the liver of mice under a number of conditions. They found that the source of this fat may be definitely ascribed to the body depots whether the fat accumulation be the result of fasting, the liver degeneration due to carbon tetrachloride vapor, or to certain extracts of anterior pituitary gland. However, when the animals are placed on a diet low in protein and other lipotropic factors but rich in carbohydrate, the fat that accumulates in the liver is not derived from the depots but probably is formed from carbohydrate of the diet. When diets rich in protein are given to animals whose depot fat contains deuterium, there is no transport of fat from depot to liver. The deuterium content of the depot fat remains constant for as long as fourteen days. No final explanation is offered for the loss of deuterium from the liver fat under these conditions.

Miyazaki (1, 2) expressed the belief that the acetone-forming capacity of the liver is somewhat influenced by its glycogen content and that extrahepatic factors may also be of considerable importance. Previous biliary obstruction or pancreatic diabetes did not influence the ability of the perfused dog liver to form acetone from butyric acid. Poisoning of the liver with phosphorus, phenylhydrazine, toluylenediamine, carbon tetrachloride or chloroform, however, diminished this ability.

Ardy & Gallo found an enormous increase in the reducing power of aseptically autolyzing liver in depancreatized dogs which



were treated with massive doses of choline. Livers from depancreatized dogs which were not treated with choline failed to show such a rise in reducing power on incubation. Since the fat content does not seem to be materially affected under these experimental conditions, it is concluded that this extra reducing substance does not come from fat.

Marks & Young found that young and adult rabbits receiving two injections of glycotropic anterior pituitary extracts during a twenty-four hour fast showed higher liver and muscle glycogen levels than the controls fasting for the same period. The subcutaneous injection of one unit of insulin to young rabbits, treated during a twenty-four fast with a thyrotropic or prolactin preparation, was not followed by a rise of liver glycogen. Those treated with a crude saline extract of the anterior lobe of the hypophysis during the fast showed a high level of liver glycogen, which was raised still further by the injection of one unit of insulin. The livers of the young rabbits receiving injections of the thyrotropic preparation during the fast contained over eleven times as much glycogen as the livers of control animals which had received no injection.

Blixenkrone-Møller (1) concluded from studies of isolated perfused cat livers that the liver functions so as to provide constantly *d*-glucose and ketone bodies to the blood stream to be used as energy sources by muscle and other tissues. The respiratory quotient of livers from fasted cats averages 0.57, from depancreatized cats 0.37. A partial explanation of the low respiratory quotient is found in the sometimes demonstrable desaturation of fat acids and in the formation of glucose from non-carbohydrates, but the most important factor is the formation of ketone bodies. Livers of the depancreatized cats and of cats treated with epinephrine or phlorizin form much more ketone bodies than normal. Comparison of oxygen consumption and formation of ketone bodies shows that one molecule of fat acid yields up to four molecules of ketone bodies instead of the usually assumed one. In general, the formation of ketone bodies increases with the fat content of the liver and decreases as the glycogen content increases.

In a second paper Blixenkrone-Møller (2) reported on studies of formation of carbohydrate and ketone bodies from acids in artificially perfused cat livers. He found that fat acids with an even number of carbon atoms are transformed to four-carbon

chains in the perfused liver. By  $\beta$ -oxidation this yields ketone bodies and by  $\gamma$ -oxidation succinic acid which can be changed to *d*-glucose via pyruvic, fumaric, malic and oxalacetic acids. Butyrate added to the perfusing liquid yields twenty per cent of ketone bodies and the remainder is converted to carbohydrate. The *d*-glucose-nitrogen ratio may rise to about twenty. Addition of ketone bodies does not increase the carbohydrate, but added succinic acid increases carbohydrate considerably, giving a ratio of *d*-glucose to nitrogen as high as 42. Addition of caprylic acid lowers the respiratory quotient and two molecules of ketone bodies are formed from each molecule of caprylic acid.

Deuel, Hallman & Murray expressed the belief that ketolysis rather than antiketogenesis accounts for the action of carbohydrate on ketonuria. They found that alcohol had no effect in decreasing ketonuria in fasting rats, whereas *d*-glucose brought about almost complete abolition. The amount of *d*-glucose necessary was equivalent to only 1.6 per cent of the total fat metabolism.

#### ARGINASE

Lightbody found that characteristic variations in the concentration of arginase in the liver of white rats occurred before sexual maturity was reached and were common to both sexes. A wide divergence between the sexes followed sexual maturity, appearing at sixty to seventy days and reaching a maximum at ninety to 110 days, when the livers of the female rats contained sixty per cent as much per unit of weight as did those of the males. Decreases occurred during middle life and persisted in old age. The concentration in the livers of rats of both sexes aged 400 to 550 days was the same as in the young rats before sex differentiation.

#### VITAMINS

Thorbjarnarson & Drummond found that the storage of vitamin A in the liver was facilitated by the presence of fat in the diet. Bauereisen indicated a greater storage of glycogen in the liver due to the inclusion of vitamin A in the diet.

Elvehjem, Madden, Strong & Woolley found that the entire activity of liver in supplying the anti-blacktongue factor may be accounted for by its potential supply of nicotinic acid. Harvey, Smith, Persons & Burns, however, by testing the activity of various fractions of liver in producing a cure of canine blacktongue

concluded that two substances partially separable by the method of fractionation are present in the liver. Both are necessary for the elaboration of some compound which is potent in the cure of experimental blacktongue in dogs.

Lipschitz, Potter & Elvehjem found that the ability of the liver to metabolize pyruvic acid was restored by oral administration of *d*-glucose to fasting birds. In polyneuritic birds similar feeding of *d*-glucose caused deposition of liver glycogen and increased the ability to remove pyruvate, although normal values were not obtained.

Smith, Warner, Brinkhous & Seegers found that the plasma prothrombin eventually falls to low levels in dogs with a biliary fistula and bleeding commonly occurs. Faulty absorption of vitamin K from the intestine is an important causative factor. Feeding bile permits absorption of vitamin K and there is a slow rise in the prothrombin level. If large amounts of vitamin K are given and bile or bile salts supplied, there is a rapid rise. Supplements of vitamins A and D did not correct the prothrombin deficiency in these animals. Brinkhous, Smith & Warner found that the bleeding tendency so often seen in patients having obstructive jaundice or biliary fistulas is due to a deficiency in plasma prothrombin. This deficiency is related in part at least to absence of bile in the intestine and is relieved by bile feeding. The beneficial effect of bile feeding was greatly enhanced by supplementing the bile with fat-soluble alfalfa extract, rich in vitamin K. In an earlier paper Smith, Warner & Brinkhous had reported that the bleeding tendency in acute chloroform intoxication of dogs was due to deficiency in both plasma fibrinogen and plasma prothrombin. A much greater decrease occurred in the plasma prothrombin with minor degrees of hepatic injury and there is indication that the liver is concerned in the manufacture of plasma prothrombin. Greaves & Schmidt found that rats with a biliary fistula lost the coagulability of the blood because of a decrease in the prothrombin level, which was restored by the administration of vitamin-K concentrate. Dam found that liver and liver fat, as well as cabbage, alfalfa, spinach, tomatoes, and other foods, contained vitamin K, which prevented hemorrhagic disease of chickens, geese and other fowl when this fat-soluble factor was deficient or absent from the diet. Thayer, McKee, MacCorquodale & Doisy reduced this prolonged clotting time by the administration of minute amounts of

vitamin-K concentrates. The crystalline compound with vitamin-K activity has been isolated by Thayer, MacCorquodale, Binkley & Doisy.

Almquist observed that the erosions of the gizzard which develop when chickens are fed a deficient diet are independent of the vitamin-K content of the diet but that bile, cholic acid, desoxycholic acid, sodium glycocholate and sodium taurocholate added to the diet prevent the development of erosions of the gizzard. Heymann showed that vitamin D is not absorbed unless bile is present in the intestine. This vitamin is excreted in rather large amounts in the bile of dogs with biliary fistula. The administration of desoxycholic acid was also shown by Greaves & Schmidt (1) to increase the absorption of vitamin E when given orally to rats with a biliary fistula.

#### HEPARIN

Best, Cowan & Maclean studied heparin and the formation of white thrombi and found that when a shunt composed of glass or cellophane tubing is inserted between an artery and a vein in anesthetized dogs, cats, monkeys or rabbits, large white thrombi rapidly form and, in many cases, completely obstruct the flow of blood. A large dose of heparin usually prevents or delays the formation of these thrombi in dogs, cats or monkeys. In rabbits growth of the thrombi is apparently inhibited but very small clumps of platelets may form. The clinical use of heparin in preventing thrombosis is discussed in reviews by Best and by Murray & Best. Wilander by special staining reactions observed that heparin was present in the mast cells, particularly those in the vessel walls, the liver and the lung, and expressed the belief that the site of origin of heparin is in these localities.

#### PLASMA PROTEINS

Madden, George, Waraich, Whipple & Sullivan found that when blood-plasma proteins are depleted by bleeding with return of the washed cells (plasmapheresis), it is possible to bring dogs to a steady state of hypoproteinemia and a uniform plasma protein production on a basal low-protein diet. These dogs are clinically normal, with normal appetite, no anemia and normal nitrogen metabolism. The normal dog (10 to 13 kg. body weight) has a

substantial reserve store of plasma-protein building material (10 to 60+ gm.), which requires two to six weeks of plasmapheresis for its complete removal. After this period the dog will produce uniform amounts of plasma protein each week on a fixed basal diet. Dogs previously depleted by plasmapheresis and then permitted to return to normal during a long rest period of many weeks, may show much higher reserve stores of protein-building material in subsequent periods of plasma depletion. Under uniform conditions of low-protein diet intake when plasmapheresis is discontinued for two weeks the plasma protein-building material is stored quantitatively in the body and can subsequently be recovered in the next two to three weeks of plasmapheresis. Given complete depletion of plasma protein-building reserve stores the dog can produce very little (about 2 gm. per week) plasma protein on a protein-free diet; this may be related to the wear and tear of body protein and conservation of these split products. Abscesses produced in a depleted dog during a fast may cause some excess production of plasma protein, which is probably related to products of tissue destruction conserved for protein anabolism. Gelatin alone added to the basal diet causes little plasma protein production but when supplemented by tryptophan gives a large protein output, while tryptophan alone is inert.

Chanutin, Hortenstine, Cole & Ludewig studied blood plasma proteins in albino rats after partial hepatectomy and laparotomy. Sixty to seventy-five per cent of the total liver tissue was removed from some animals while others were subjected to laparotomy alone, during which the liver was handled and the intestines were exposed for a period similar to that occupied by partial hepatectomy. On the first day after partial hepatectomy blood plasma fibrin, albumin and globulin were decreased and the albumin concentration remained depressed until the fourth week after operation. The fibrin and globulin concentrations increased on the second and third days and remained above the normal values for the thirty-day period of observation. After laparotomy there was an immediate increase in the fibrin and globulin content and a decrease in albumin. The globulin remained high throughout the period but after about the eighteenth day the albumin and fibrin concentrations approached the normal value. The most important effect of tissue injury and reduction of liver tissues is the prolonged reduction of the plasma albumin accompanied by increased glob-

ulin concentration. Hepatic insufficiency might possibly account for the decreased albumin but not for the increased globulin.

Daft, Robscheit-Robbins & Whipple found that dog plasma given by vein to a protein-fasting dog was well utilized under certain conditions and would maintain the dog in nitrogenous equilibrium. There was no wastage of surplus urinary nitrogen in after-periods. It appeared that the introduced protein was utilized efficiently in body metabolism to replace or repair organ and tissue protein. This mechanism is operative under normal as well as emergency conditions and suggests a "dynamic equilibrium" between plasma proteins and cell proteins. For plasma protein to be stored or utilized in the liver, muscles or body tissues, it must be stored as such or slightly catabolized (large aggregates rather than amino-acids) and synthesized to cell protein. This mechanism may be disturbed by overloading (injection of too much plasma) or lack of adequate carbohydrate and fat.

Howland & Hawkins observed the conversion of part of plasma protein to sugar when plasma protein was fed to phlorhizinized dogs. When plasma protein is injected intravenously no sugar is formed and there is no excess nitrogen in the urine. It is suggested that plasma protein is removed from the blood in rather large aggregates of amino-acids which are then reassembled by the cells to form body protein. It is not necessary that protein be completely catabolized before being elaborated into specific body proteins. Cantarow observed a rapid diminution of the proteins of the blood immediately after paracentesis and the removal of large amounts of protein with the ascitic fluid. Thompson, Ravdin & Frank observed retardation in healing of wounds associated with hypoproteinemia in dogs, which could be averted by restoration of the serum protein to normal. Lyophilized serum or plasma was useful in the restoration of the concentration of blood proteins.

#### WATER AND MINERALS

Fiessinger, Gajdos & Panayotopoulos determined the conductivity, plasma and cellular chlorides, and serum proteins in the blood of the portal vein, vena cava and femoral artery. After the administration of water by mouth there was first a dilution of the portal blood and later distention of the liver. Still later there was transference of the water to the general circulation. An an-

imal with an Eck fistula did not show consistent results in that the blood was not generally diluted after the administration of water. When the liver is poisoned with phosphorus (rabbits), the blood is not diluted after water for one hour, which indicates injury to the liver. There appears to be a definite tissue factor in the liver which regulates the transference of water and the likelihood of diuresis.

#### POISONS

De Saram in a study of the role of the liver in atropine disposal found that rabbits tolerate much larger doses of atropine and store greater amounts in their livers with intraportal than with ear-vein administration. Atropine is not excreted in the bile and little is actually destroyed in ground-up liver tissue. A great deal of the stored atropine can be easily washed out from the vascular system of the liver. It is suggested that disposal takes place by temporary union with the vessel walls, reabsorption into the blood stream and slow excretion by the kidneys. There appears to be little evidence in favor of a specific detoxifying action of the liver against atropine.

Bodo, CoTui & Benaglia in studying the mechanism of morphine hyperglycemia found that the slight hyperglycemia obtained with morphine in adrenal-inactivated dogs and cats is not abolished after liver denervation or abdominal sympathectomy. After removal of both lateral sympathetic chains (complete sympathectomy) morphine causes either slight hypoglycemia or no change. Sympathin produced by morphine is the agent responsible for the slight hyperglycemia observed in animals with (1) inactivation of the adrenals; (2) inactivation of the adrenals and denervation of the liver and (3) abdominal sympathectomy respectively. Morphine in the doses given did not act on the liver cells directly to cause a breakdown of liver glycogen and it did not suppress the secretion of insulin. It did not bring about secretion of epinephrine by acting on the denervated adrenal glands directly and it did not act on the sympathetic nerve endings and collateral sympathetic ganglia. Morphine in producing sympathin must act on the lateral sympathetic chain, the spinal cord or the supraspinal centers.

Scott & Fisher fed large quantities of zinc to cats. The amount of zinc deposited in the liver was from seven to fifteen times as



great as that found in the control animals. Von Glahn, Flinn & Keim administered various arsenates to rabbits, which caused necrosis of the liver. A diet rich in carbohydrate appeared to protect the liver against the injurious effect of the arsenates. Aubertin, Lacoste, de Lachaud & Martinet found that mild poisoning with phosphorus and with carbon tetrachloride in dogs decreased the blood fibrinogen and the albumin of the plasma. Cantarow, Stewart & Morgan injected carbon tetrachloride subcutaneously into adult cats in doses which caused death of one-third of the animals within four days. A group of cats given calcium gluconogalactogluconate daily after the administration of carbon tetrachloride showed much less impairment of hepatic function and fewer histologic changes than the untreated animals. Regeneration of the necrotic liver appeared complete in about twelve days but during the third or fourth week after injection of carbon tetrachloride there appeared a secondary regressive phenomenon in which degeneration of about one-half of each lobule again occurred.

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DIVISION OF EXPERIMENTAL MEDICINE  
THE MAYO FOUNDATION  
ROCHESTER, MINNESOTA

## BLOOD: PHYSIOLOGY OF FORMED ELEMENTS AND PLASMA; BLOOD CLOTTING\*

By J. HAROLD AUSTIN

*John Herr Musser Department of Research Medicine  
University of Pennsylvania, Philadelphia, Pennsylvania*

### REVIEWS

An annual review of literature on hematology for 1937 by Bethell, Isaacs, Goldhamer & Sturgis (1) occupies ninety pages and covers 482 contributions, only about fifty of which are among the 246 papers reviewed in this more restricted chapter.<sup>1</sup> Eagle (2) has reviewed coagulation; Silberberg (3), thrombosis; Isaacs (4), the formation and destruction of red blood cells; Hahn (5), the metabolism of iron; Heath & Patek (6), the anemias of iron deficiency; Reich (7), the relation of endocrines to blood disorders; Evans (8), the anemias of pregnancy; Tocantins (9), the origin, morphology and function of platelets.

### BLOOD COAGULATION

*Prothrombin.*—The hemorrhagic diathesis induced in chicks when given a diet deficient in Dam's vitamin K, the hemorrhagic state developed in rabbits fed spoiled clover hay (10), and the hemorrhagic tendency associated with obstructive jaundice (11), with experimental liver injury (12), and in the newborn (13), all are characterized by diminished prothrombin in the plasma. These conditions respond to alfalfa, or a fat-soluble extract from alfalfa rich in vitamin K. The effective dietary principle has been isolated in crystalline form (14) and may under certain conditions be produced by bacterial activity (15), or, in laboratory mammals but not in fowls, perhaps by intestinal synthesis (16). The effective principle has also been found in hog-liver oil, cabbage, spinach, tomatoes and fish meal (15, 17). Bile salts in the intestines favored its absorption (11, 18). There is no evidence in chicks that the prothrombin deficiency is due to an inactive bound form of prothrombin. Evidence suggesting a difference between prothrombin

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<sup>1</sup> This review is based on literature published between January 1, 1937 and June 30, 1938 with a few additional papers appearing during July and August 1938.

of ducks, pigeons, and chicks on the one hand and of rabbits, dogs, and man on the other hand has been published; prothrombin was not found identical with complement (19). The method for prothrombin assay requires cautious interpretation (20). In obstructive jaundice the heparinized plasma required more tissue extract than normally to induce coagulation within standard time; this was not the case in thrombocytopenic purpura nor in hemophilia (21).

Thrombin could not be replaced by "tissue fibrinogen" for the coagulation of purified blood fibrinogen in the presence of calcium (22).

*Calcium and coagulation.*—When the calcium concentration in plasma fell below 2.5 mg. per 100 cc. retardation of coagulation was noticeable (23). At pH 7.0 to 8.0 the coagulation of diluted citrated plasma ceased at calcium ion concentrations below 0.28 to 0.35 mM per l. in man or 0.18 to 0.24 in dogs; the maximally effective calcium ion concentration was 1.25 mM per l. A shift of pH beyond 7.0 to 8.0 increased coagulation time and more so as the calcium-ion concentration was lowered (24). The removal of calcium by electrodialysis from a mixture of prothrombin, cephalin and calcium reduced the activity of the resulting thrombin only when the calcium was removed immediately after its addition (25). For maximal rate of activation of prothrombin to thrombin, the ratio of calcium to prothrombin was of primary importance; the cephalin concentration had an important but secondary influence. Excess of either calcium or cephalin was inhibitory. The intermediary step appeared to be not a compound of calcium and cephalin but a colloidal complex of prothrombin, cephalin and calcium (26). At 38° C. activation was accelerated but so also was the irreversible conversion of thrombin to inactive metathrombin.

*Thromboplastin.*—Preparations of thromboplastin from certain species were more active with the prothrombin from the same species than with that from other species (27). The observation that crude or crystalline trypsin (28) and five out of seventeen venoms tested (29) could be substituted for a mixture of calcium and platelets or of calcium and tissue extract for activating prothrombin *in vitro* has been advanced as support of the view that the activation of prothrombin involves proteolysis.

*Heparin.*—Best and his associates (30, 31, 32) have repeatedly injected their highly purified, non-toxic preparation of heparin



intravenously into animals and 222 patients. They have thus kept coagulation time retarded for days and have reduced the incidence of venous thrombosis and of white thrombus formation on injured intimal surfaces or on glass tubes inserted in the circulation.

Ten times as much heparin was required to inactivate thrombin as was needed to inhibit the activation of prothrombin; the inhibition by heparin of activation of prothrombin was counteracted by increasing the concentration of cephalin (33). The normal antithrombin of blood appeared to be an albumin-like substance which will not bind thrombin while fibrinogen is present. Addition of heparin increased the affinity of this albumin for thrombin. If the albumin had been inactivated by heating to 67° C. addition of heparin produced no antithrombin effect; hence heparin is not itself an antithrombin (34). The action of heparin was blocked by the protamine, salmine, given intravenously, as was the anticoagulant action of cellulose sulfuric acid (35).

*Other anticoagulants.*—Other anticoagulants that have been investigated include hydrochloric acid, various organic acids, diethylamine (36), carbon dioxide tension (243), a series of basic organic compounds (37), dyes (38 to 42), polysaccharides (43), the rare earths (43, 44) and the thio-aureic compounds (45). Most of these inhibit the activation of prothrombin. In anaphylactic shock in the rabbit and dog the antithrombin titer has been found one hundred times normal, with fibrinogen and prothrombin concentration normal (46).

*Fibrinogen.*—The action of thrombin on fibrinogen is reported to be its conversion to a soluble substance, named "profibrin", which can be distinguished from fibrinogen by its response to salting out and to adsorbents (47). The further change to fibrin network is considered a reversible physical change, not requiring the intervention of thrombin. Profibrin promoted the agglutination of platelets and formation of white thrombi. Denaturation of fibrinogen by various agents, including heat, occurred by way of an intermediate substance resembling profibrin (48). A concentrated solution of urea inhibits the coagulation of fibrinogen (49).

*Hemophilia.*—Crystallin trypsin, placental extract, or fresh normal serum have induced clotting of either normal or hemophilic oxalated plasma; however, a larger dose of hemophilic serum was required for this purpose (50). A globulin fraction from normal plasma promoted the coagulation of hemophilic blood both *in vitro*

and, when given intravenously or intramuscularly, *in vivo*; the corresponding fraction from hemophilic plasma lacked this property. Yet these fractions from the two sources had the same prothrombin titer (51, 52). Injection of the fraction from normal plasma into the hemophilic shortened coagulation time with return to the initial value in 24 hours, after which repetition of injection was again effective; however, reinjection during the period of shortened coagulation time, although it increased in the plasma the substance which promotes clotting, did not delay the return of coagulation time to the initial value (53). A globulin substance from citrated cell-free beef plasma has similar properties; applied locally as a dried powder it is an effective hemostatic in hemophilia (71).

Two cases of hereditary pseudo-hemophilia have been reported; both males and females exhibit and transmit the disease (231).

#### BLOOD VOLUME

A micromethod for the determination of blood volume in rats (54), and refinements in clinical measurements using "Evans Blue" dye (55) and a photoelectric colorimeter (56) have been described.

Normal values on 49 human males and 41 females indicated that the blood volume decreases directly with metabolic rate and with vital capacity as age advances (57). As dogs of increasing weight from five to thirty kilos were studied, the plasma volume increased from 41 to 52 cc. per kilo, with increase also in cell volume and total blood volume per kilo (58).

Continuing plasmapheresis accompanied by prompt return of the cells suspended in saline proved fatal in dogs when, within  $4\frac{1}{2}$  to 20 hours, plasma amounting to from 2.8 to 5.6 per cent of the body weight had been removed; the final hemoglobin concentration ranged from 121 to 168 per cent (59). When, however, a gum saline solution was used in which to suspend the replaced cells, the serum protein could be washed until mere traces remained and the animal still survive; rapid regeneration of serum albumin occurred, and serum globulin reached initial concentration in about 10 days (60).

Small changes in blood volume have been reported following ingestion of water (61), after mercurial diuretics (62, 63), after intravenous injection of ammonium chloride or ammonium hydroxide (64), and after vitamin-B<sub>1</sub> administration (65). Larger

changes have been measured in rabbits after intravenous injection of acids, alkali, saline, glucose, gum solutions and blood (66), and in patients when 1000 cc. of saline or glucose were given intravenously in a half hour (67). Rise of hemoglobin and cell count to a maximum 24 hours after blood transfusion has been attributed to loss of the injected plasma (68). In a study of sixteen major operations the trauma was found to be accompanied by a fall of plasma volume (maximal decrease, 34 per cent) and a rise of interstitial fluid volume estimated with thiocyanate (maximal increase, 45 per cent); that no commensurate change in hemoglobin or plasma-protein concentration occurred was a striking observation (69). During rapid recovery from congestive heart failure, marked increases in packed red cells, hemoglobin and plasma-protein concentration within three to six days were interpreted as evidence of shrinkage of plasma volume amounting to 25 per cent or more (70).

*Plasma-protein production.*—When serum-protein regeneration in the dog was studied by daily plasmapheresis sufficient to maintain the serum protein at four per cent there was, during the initial weeks, a faster regeneration suggesting a reserve of potential serum protein (72). When plasmapheresis was discontinued this reserve was rebuilt to an even higher level than the initial. After 25 to 30 weeks of daily plasmapheresis, however, impaired serum-protein regeneration, skin lesions and susceptibility to infection were observed. The rate of regeneration was depressed by a sterile abscess, by digestive disturbances and by liver injury (73). The efficiency of various amino acids added to the diet was measured (72, 74).

Melnick & Cowgill (75), using a similar technique, recognized no change in rate of protein regeneration during the first sixteen weeks of daily plasmapheresis provided the dog was furnished what they considered to be an adequate protein ration. Endogenous sources supplied serum protein at 21 per cent of the maximal rate of regeneration. The rate of regeneration was lower during pregnancy or lactation (76). It was inferred (77) that some specific mechanism responsible for production of serum protein must be disordered in the hypoproteinemia of at least some cases of disease and that lack of protein in the diet and loss of protein in the excretions is not in general an adequate explanation. Study of the hypoproteinemia of portal cirrhosis has led to the same conclusion (78). The rate of serum-protein regeneration has been studied after

renal or hepatic injury, after thyroidectomy, after splenectomy (79) and after complete removal of serum protein by replacement with a gum-saline suspension of blood cells (60).

*Intravenous injection of colloids.*—When dog-plasma protein was given intravenously to dogs it was utilized to maintain nitrogen balance (80), the existence of a dynamic equilibrium between the protein of plasma and tissue being thereby suggested; an intoxication which occasionally occurred was relieved by abundant fat and carbohydrate in the diet. Redissolved dried serum (81) and human ascitic fluid (82) have been used successfully to replace plasma. Among the effects of injecting gum-acacia solution (83) has been noted a fall of serum protein due less to dilution than to absolute decrease of circulating protein (84); after ten to twenty-one days the total circulating protein was still low but normal concentration had been restored by loss of plasma volume.

#### BLOOD CELLS

The various types of blood and bone-marrow cells and their genetic relationships have been studied in the embryo, foetus and in early post-natal life (85, 86, 87) and by tissue culture (88, 89, 244) especially as applied to bone marrow (90, 91). The origin of the monocyte has been investigated (88, 92, 93). The ratio of erythroid to myeloid activity was found higher in the tibia than in the marrow of other bones of monkey and man (94). Other studies during 1937 of types of blood cells and their relationships have been recently reviewed (1). The spectroscopic absorption of free hemoglobin is reported different from that of the erythrocyte; heating a mixture of stromatin and free hemoglobin at 37° C. altered the spectroscopic absorption from that of the former to that of the latter (95).

Sympathetic, parasympathetic, and endocrine control have been reported with respect to bone-marrow activity, hemoglobin concentration and reticulocyte count (96 to 102). The usual rise in red cell count following epinephrin was absent in cases of anemia, but occurred following splenectomy (103).

*Response to diet.*—Erythrocyte production was depressed less than was the general growth rate of the young albino rat by deficient caloric or inadequate lysine content of the diet (104). On a diet lacking some constituent of the vitamin-B group, dogs responded to the feeding of indole with a hemolytic anemia (105)

and became abnormally sensitive to phenol or amidopyrine (106, 107). Young rats on a vitamin-B deficient diet were abnormally sensitive to cold environment and became anhydremic (108). Deficiency of some vitamin-B<sub>2</sub> constituent of Peter's eluate induced in rats aplastic anemia, agranulocytosis, thrombocytopenia, myeloid depression and hemorrhagic manifestations (109); the marrow changes were prevented by administering nicotinic acid (110). No benefit followed the use of nicotinic acid in pernicious, macrocytic or hypochromic anemia or in myeloid leukemia (111). A factor favoring the maturation and discharge of cells from the marrow of the guinea pig was apparently deficient in a diet of oats, carrots and lettuce, but adequately supplied in hay or in liver extract (112). A protein-deficient diet during pregnancy in the rat induced a megaloblastic marrow and macrocytic anemia (113). Yeast vitamin-B (114), carotene (115) and vitamin-C (116, 117) therapy have been credited with favoring red cell regeneration.

The dog, anemic from bleeding, utilized for hemoglobin production orally administered hemoglobin of the dog, goose or sheep (118). Globin from the horse or dog given intravenously to the anemic dog was usually toxic, but occasionally was utilized completely in new hemoglobin production; given orally it was a better source of hemoglobin than was liver protein (118). In the presence of a biliary fistula the power of the dog, anemic from bleeding, to regenerate hemoglobin was impaired and iron and liver given orally had only half their usual effect, although they were fully effective when injected intravenously (141). Aetioporphyria fed in cod-liver oil to rabbits anemic from bleeding favored hemoglobin production (104).

*Iron and hemoglobin production.*—A diet deficient in iron induced in young rats a microcytic polycythemia, the effect increasing when the diet was continued through successive generations; therapeutic response to iron was favored by furnishing traces of copper (119). In young rats anemic from a milk diet, iron favored both hemoglobin and erythrocyte production (120) and was more effective given intraperitoneally than by mouth (121).

Of retained dietary iron only a part is utilized for hemoglobin production. The fraction has varied from almost none to perhaps sixty per cent as a maximum (122 to 127). The dipyriddy method of measuring so-called "available iron" does not measure the

amount which will be utilized in hemoglobin formation (127). Chlorophyll iron favored hemoglobin regeneration in the anemic dog (115).

During the rapid fall of hemoglobin concentration which occurs during the first ten weeks of infancy about two-thirds of the released iron was stored (128).

The recent literature concerning the dose of iron required for full therapeutic effect has been lately reviewed (1); three other reports may be noted (125, 129, 133). Lowered gastric acidity (130, 131) or large doses of mucin (132) impair the absorption of iron given orally, and infection depresses its effect on rise of hemoglobin (133).

*Other metals.*—Favorable effects upon the response of the anemic subject to iron therapy when traces of copper are given orally are reported for rats (119, 134), dogs (115) and infants (133). Others recognize no benefit from copper as a supplement to iron (121, 130, 135, 136). The normal range for blood copper has been reported for boys and girls (137). Cobalt (2 mg. per kg. per day) induced in dogs a rise of red cell count with marked reticulocytosis (138). This effect of cobalt was counteracted by feeding liver or by liver extract intramuscularly but not by ventriculin nor by liver extract heated during its preparation (139). This effect of cobalt was not noted in the dog when the dose was only 0.8 mg. per kg. per day (140).

*Liver extracts.*—That appropriate liver extracts supplement iron in favoring recovery from hypochromic anemia has been claimed (120, 142) and also denied (136). The primary undetermined factor in liver extract responsible for relief of pernicious anemia was augmented by at least three accessory factors, *l*-tyrosine, a complex purine and a peptide (143). Livers from patients with aplastic anemia had a normal content of the principle effective in pernicious anemia (144).

*Gastric factor.*—Normal human gastric juice, containing the intrinsic factor, reacted at pH 7.4 with casein to increase the trichloroacetic acid soluble nitrogen but without increase of amino nitrogen; both the intrinsic factor and this special proteolytic reaction with casein persisted after pepsin had been removed (145). Removal of caseinogen from milk by acid precipitation rendered the milk, when mixed with normal gastric juice, incapable of inducing a reticulocyte response in pernicious anemia (146). Beef

muscle and normal gastric juice interacted at pH 5 or 7 but not at pH 1.8 or 2.5 to form a substance effective in pernicious anemia; the substance was less thermostable than the effective principle in liver extract (147). The intrinsic factor from hog stomach and from normal human gastric juice was precipitated at half-saturation with ammonium sulfate and was then not dialyzable (148). The property which normal human gastric juice is reported to have of augmenting the effect of liver extract upon incubation with it, persisted after removal of pepsin, rennin and mucin or half-saturation with ammonium sulfate but was removed by saturation with ammonium sulfate (149). Reported effectiveness of desiccated duodenal mucosa of swine (150) and of saliva (151) in inducing a reticulocyte response in pernicious anemia has suggested a wider distribution of the intrinsic factor. Injection of concentrated gastric juice into pregnant rats was followed by changes in the number and structure of the foetal erythrocytes (152); but injection of liver extract into pregnant rats produced no effect on the mean red cell diameter of the newborn rats (153).

The atrophy of the gastric mucosa in pernicious anemia has been studied gastroscopically (154) and histologically (155). Macrocytic anemia has been reported following resection of the pyloric end of a pig's stomach (156), in some cases of gastric carcinoma (157, 158), in chronic pancreatitis or carcinoma of the pancreas (159) and after enteroenterostomy (160). Hypochromic microcytic anemia with changes in skin and nervous system developed in young swine after extirpation of the ventriculus and the part of the duodenum containing Brunner's glands (161).

*Physiologic variation in hemoglobin concentration and erythrocytes.*—Hemoglobin and erythrocyte concentration in the blood have been studied in the chick from the ninth day of incubation to the third day after hatching (162), in pigeons during the two months after hatching (241), in rats from birth to three months (119), in infants and children (128, 133, 163), in negro and white infants (164), in adolescents (137), and in adults of the United States (165) and of India (166, 167, 168).

Diurnal variations of hemoglobin and red cell count (169, 170) and changes during pregnancy (171) and the menstrual cycle of women (170) and of monkeys (172) are reported. Venous and capillary values were found different only in early infancy (173).

Nine new cases showing Dresbach's elliptical erythrocytes



appearing in four generations of a family are reported (174).

*Erythrocyte longevity.*—In fifty patients anemic from a single hemorrhage from peptic ulcer and treated from the onset with a diet adequate to allow maximal regeneration of red cells, the erythrocyte count rose linearly to 4.5 millions in approximately thirty-three days; this fact does not appear to warrant the inference drawn that thirty-three days represents the average longevity of the erythrocyte (175). When bile-fistula dogs were made anemic by bleeding, there was a fall in output of bile pigment. Regeneration restored the red cell count in two to four weeks. The midpoint of this period was taken as the average starting date for the new cells. Bile-pigment excretion rose sharply at 112 to 133 days later, indicating that the average longevity of the new cells was 124 days (176). After transfusing anemic subjects of an appropriate blood group with cells compatible but of a different group, the native cells were from time to time agglutinated from a sample of the recipient's blood and the unagglutinated transfused cells were counted; the survival time in seven cases for the transfused cells was forty to eighty days (177).

*Hemolysis and fragility.*—Hemolysis of the erythrocyte in hypotonic solutions occurred when the cell had swollen to spherical form without change in surface area and had thus attained a "critical volume." The increase in volume possible depended on the initial shape and this determined the tonicity of the solutions required to induce hemolysis (178, 179, 180). The decreased fragility of guinea-pig erythrocytes after splenectomy was associated with an increase of their "critical volume" at hemolysis (181). Phagocytosis of erythrocytes and of granulocytes in the spleen normally and after injection of homologous blood has been studied (245). The erythrocytes of even such closely related species as the albino rat and the albino mouse exhibited strikingly different rates of hemolysis when subjected to each of a variety of hemolytic agents (182). Racial strain (183), exercise and subjecting the animal to external heat (184) altered erythrocyte fragility. Precautions advisable in the clinical measurement of fragility are described (185, 186).

Erythrocytes from cases of pernicious anemia in relapse were less resistant than normal to saponin hemolysis (187) and their permeability to dextrose was four times normal (188). Their rate

of disintegration during relapse was highly variable; in remission it became normal (189).

The hemolysis which tends to follow injection of pituitrin into rabbits has been attributed to an associated hydremia with hypotonicity of the plasma (190).

Ether extracts of human serum had hemolytic properties which were inhibited by whole human serum; this antihemolytic effect of whole serum was less evident in the serum of the infant (191).

From normal human and pig blood an ether-insoluble fraction of concentrated protein-free plasma has been obtained which depressed blood-cell destruction in certain hemolytic anemias; it is suggested that in sickle-cell anemia and perhaps in hemolytic icterus a substance is lacking from the plasma which normally represses hemolysis (192).

A hemolysin which has been found almost constantly in normal human urine was absent in free form from the urine of patients with aplastic anemia. Hemolysin appeared in these urines, however, following an acid hydrolysis which has no effect on the hemolysin of normal urine. This abnormality was not found in pernicious anemia, Hodgkin's disease or the anemia of iron deficiency (193).

In chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria, hemolysis has been induced *in vivo* and *in vitro* by slightly lowering blood pH, as from such increase of carbon dioxide tension as commonly occurs during sleep (194, 195). Six cases of acute hemolytic anemia have been described (196, 197). Three cases with sickle-shaped red cells in supposed non-negroes are reported (198, 199).

*Porphyrins and erythrocytes.*—Additional evidence supports the view that porphyrin III and porphyrin I are formed simultaneously in the body in proportionate amounts—the former, utilized in hemoglobin synthesis, the latter, excreted as coproporphyrin I in the feces (200, 201). Coproporphyrin-I excretion in the feces appears to be correlated with the rate of hemoglobin production in severe anemia, polycythemia, hemolytic icterus (202 to 205), aplastic anemia (206), alcoholic pellagra (207) and in experimental studies on dogs (208, 209). These studies indicate that in pernicious anemia in relapse there are increased rates of production and of destruction of erythrocytes.

In the chick embryo coproporphyrin I and porphyrin III

appeared simultaneously and increased together, although whether their ratio remained constant was undecided (210). High porphyrin content of young erythrocytes separated by differential centrifuging is reported (211, 212). Studies with the fluorescence microscope suggested the presence of a high porphyrin content in immature erythrocytes (213, 214, 215).

#### LEUCOCYTES

White cell counts of British aviators and Iraqi medical students in Iraq have been compared (216). The diurnal variation of white cell counts at fifteen minute intervals in six normal young men is reported (217). There was a correlation between the rise in alveolar carbon dioxide, fall of blood pH and fall of circulating neutrophiles during sleep (218). Changes in lymphocyte count in the guinea pig following various stimuli were unaltered by the removal of neutrophiles through injection of antineutrophile serum (246).

In twenty normal persons in one locality a seasonal variation in the types of circulating polymorphonuclear leucocytes was observed, with "shift to the left" maximal in January and with a rapid "shift to the right" occurring during the summer and maximal in September (219). In the leucocytes of experimentally induced peritoneal exudates increasing maturity of cell was correlated with decreasing oxygen consumption per cell; the relation of oxygen consumption to cell mass or cell surface was not determined (220).

The leucocytosis induced by epinephrin occurred in spite of splenectomy (221). Changes in leucocyte count have been noted after intraperitoneal injection of oxygen (222). The response of leucocyte count to cold, to surgical injury and to intoxication and to various drugs has been investigated (242).

In a study of the phagocytic activity of the cells in circulating normal blood and in various leukemias, the lymphocytic cells showed no phagocytosis; granulocytic cells and their precursors exhibited active phagocytosis, increasing in general as the cell became more mature; monocytic cells were actively phagocytic especially for collodion and carbon particles (223). Experiments were designed to test whether bacteria attract leucocytes chemotropically directly or only as a result of injured cells or tissues; they indicated that leucocytes can react chemotropically to substances given off directly by bacteria in the absence of tissue cells

(224). When local inflammation and necrosis were induced in the rabbit by infection with hemolytic streptococci, prolonged polymorphonuclear leucocytosis and marked marrow hyperplasia appeared to result from substances released from leucocytes undergoing necrosis and not from the necrosis of other tissues (225).

In the pig and rabbit no evidence was adduced that any type of leucocyte is important in the transfer of fat from intestinal epithelium to lacteals (226).

#### PLATELETS

This topic has been reviewed by Tocantins (9). Increase of megakaryocytes in the sternal marrow was associated with increase of circulating platelets (227) and disappearance (228, 229) or faulty maturation of megakaryocytes with thrombocytopenia (227); but such correlation has not always been found (230).

Acetone extracts from spleens of thrombocytopenic purpura when injected into rabbits induced a marked fall in circulating platelets with prolongation of bleeding time which persisted for thirty-six hours, or longer if the injections were repeated (232). Boiling decreased the potency of the extract.

That the lungs are an important source of platelets has been inferred from histological study of the lungs and because the platelet count was higher in systemic arterial blood than in systemic venous blood (233).

Seasonal variations in platelet count and differences in the count between simultaneously drawn samples of arterial, capillary and venous blood are reported (234). The platelet count in man was lowest and most constant at 7 to 8 a. m. and highest about midnight (235). The error of platelet counting or perhaps spontaneous variation at short intervals (236) makes it difficult to evaluate reported changes (237, 238, 239). Human platelets are reported to be relatively resistant to disintegration during the first four days after birth and in this period coagulation time was found to be prolonged (240).

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JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE  
UNIVERSITY OF PENNSYLVANIA  
PHILADELPHIA, PENNSYLVANIA

## HEART\*

By J. A. E. EYSTER

*Physiological Laboratory, University of Wisconsin,  
Madison, Wisconsin*

The publications considered in the present review appeared, with few exceptions, in the period from January 1, 1937 to July 1, 1938. Since it is anticipated that a review of the literature of coronary blood flow will appear next year, this topic is omitted.

### ANATOMY AND EMBRYOLOGY

The interest in recent years in aortic and carotid sinus reflexes is reflected in the appearance of a number of contributions concerning the end organs and nerve paths involved in these reflexes. Terminal end plates and fine nerve plexuses occur in the human aorta, lying mostly in the outer layer of the media (1). The peripheral receptor zones are found at the base of the innominate artery and around the mouth of the right subclavian artery (2), and are identical in histological structure with the carotid sinus receptors. In the dog, nerve fibers pass from receptors at the origin of the innominate artery to the right cardiac nerve, a nerve bundle which also contains post-ganglionic sympathetic fibers. The majority of the fibers from the left side pass into the left aortic nerve while a smaller number join a cardiosympathetic nerve running caudad between the left subclavian and innominate artery (3). In man, nerve fibers from the carotid sinus pass to the glossopharyngeal, to the nodose ganglion of the vagus and to the superior cervical ganglion of the sympathetic (2). The enlargement of the vessel that takes place at the carotid sinus results in the development of a greater tension exerted on the vascular wall in this region than in others. The walls of the carotid sinus show thinning of the media (4) and an arrangement of elastic fibers in such a way as to give greater strength to the dilated walls (5).

Receptors resembling closely those found in the aorta and carotid sinus are also present in all the veins entering the heart (6). If these, as is suggested, are the receptors for the Bainbridge cardiac accelerator reflex, it would appear that this reflex may be initiated by increase in pressure in the pulmonary veins as well as in the venae cavae.

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The epicardial surface of the mammalian ventricle contains an extensive network of thin non-medullated sympathetic fibers (7). Fine nerve networks occur in the atria and papillary muscles and an extensive interstitial plexus in the ventricles, both independent of blood vessels and degenerating only in part when the extrinsic cardiac nerves are cut (8).

Beginning specialization of the cells of the Purkinje system of the heart can be recognized in the 16.5 mm. human embryo (9). By the seventh month the node is clearly differentiated from the bundle, the latter retaining its embryonic structure for a longer period. Increase in collagen and elastic fiber elements in the mature Purkinje system occurs with increase in age. In the mature Purkinje system one may recognize large heavily medullated fibers of vagus origin (7) and spiral unmyelinated fibers forming a network surrounding the Purkinje elements (10). The latter are regarded as belonging to the sympathetic system.

The description by Schneider (11) of the incorporation of the sinus valves and other primitive structures into the superior vena cava, right atrium and coronary sinus during the embryological development of the mammalian heart and the work of Körner (12) on the structure of heart muscle striations are of interest but too extensive and detailed to warrant a brief review.

As is known to occur in the primitive cardiac tube of the chick embryo, the embryonic mammalian heart also begins to beat vigorously and regularly before it contains blood and before the circulatory system is formed (13). Cardiac muscle tissue may be maintained in tissue culture for long periods without any tendency for the cells to revert to fibroblasts (14).

The active mitosis and fiber proliferation which occur in hypertrophied hearts in children (15), appears as an exception to the widely held view that the post-natal heart increases in mass only by enlargement of fibers already formed.

#### AUTOMATICITY, RESPONSE TO STIMULATION, PERMEABILITY AND THE ACTION OF INORGANIC IONS

Spontaneous contractions occur in the isolated sino-atrial node and false tendons of the mammalian heart (16, 17, 18). The Purkinje structures respond to artificial stimulation in the same way as the myocardium. Observations pertaining to pulsations in the terminal portions of the superior vena cava in surviving mamma-

lian hearts, revives the old question as to the possibility of this region being the *primum movens* of the heart, the sino-atrial node functioning as a relay center (19).

The left atrium of the rabbit isolated in nutrient solution, often shows no rhythmic activity, and offers a favorable preparation for certain studies concerned with the effects of artificial stimulation. It is known that with increasing rates of rhythmic stimuli an "optimal rhythm" can be found at which the heart amplitude is at a maximum. The onset of this maximal response is sudden (20). If the stimulation rate required to produce the optimal rhythm is determined at different temperatures (21) the rate as a function of temperature accords with the exponential relation of van't Hoff-Arrhenius. On the other hand the duration of contraction as a function of temperature follows the power function of Bèlehrádek. Amplitude, duration of contraction and optimal rhythm, all have different temperature optima. The minimum interval between successive stimuli to which a quiescent amphibian heart will respond with a complete contraction, is about three times as long for the ventricle as for the auricle (22). If in either case the stimulation interval is still further shortened, alternans, periodic responses or "half rhythms" result.

Observations concerning the automaticity and rôle of inorganic ions in the activity of invertebrate hearts are reported for the snail heart (23, 24), the insect heart (25), and the heart of the oyster (26). It appears that in the oyster heart, potassium excess leads to a standstill in systole, while calcium excess slows the heart and causes ultimate standstill in diastole, these results being opposite to the well known action of these ions in the amphibian heart. The chick embryo heart responds similarly to the adult heart (27). The snail's heart shows selective permeability to potassium and calcium, the former tends to diffuse from within outward, the latter in the opposite direction. Magnesium appears to pass equally well in both directions (28). The effects of an- and catelectrotonus on rate and tonus are reported in the mussel heart (29) and in the heart of the snail (30).

The application of an anaesthetic agent locally to different parts of the frog's heart, in sufficient concentration to abolish irritability to artificial electrical stimulation, may not affect the initiation and conduction of the cardiac impulse, according to the report of Witz (31).



Humoral regulation of the heart beat is the subject of several reports. Demoor, the principle advocate of this theory in recent years, reaffirms the specificity of the substances present in extracts of the sino-atrial node (32). The isolated left atrium of the rabbit, which is ordinarily quiescent or shows disordered or grouped contractions, develops a regular rhythm of large amplitude when an extract containing the "*substances actives*" is added. Before this addition, the preparation is insensitive to the action of epinephrine and acetylcholine; after the addition it becomes extremely sensitive to both. By heating the extract it may be shown that there are at least two active components: one, thermolabile, which causes the rhythmic beat and sensitizes the atrium to epinephrine and acetylcholine; the other, thermostable, which increases the beat inaugurated by the thermolabile component and acts in general like epinephrine. Hermann, Cornut & Guiran (33) on the other hand, using the same preparation, report that epinephrine is equally as effective as extracts of nodal tissues in converting the quiescent atrium or one showing a disordered beat into one showing rhythmic contraction. Iwao (34) reports that histamine promotes automaticity in the isolated ox heart and in strips from the same, and that the histamine content is much greater in the sino-atrial node and right atrium than in the non-automatic muscle of the left atrium and ventricles. He expresses the view that the effects of tissue extracts reported by other workers are explicable on their histamine content.

#### MECHANICS

Attempts to correlate various mechanical factors of the circulation appear in further elaboration of the "*Windkessel*" or elastic reservoir theory, relating stroke volume, heart rate, elastic resistance of the vessels and blood pressures (35), and the "*Seperator methode*", in which intraventricular and intra-arterial pressure and ventricular volume are related mathematically by the use of amplitude-frequency products (36).

Changes in inflow and outflow of blood during the cardiac cycle produce pulsating air currents in the upper respiratory passages (*Atempuls*) which may be recorded when movements due to respiration are suppressed. Records from dogs show that various mechanical events in the heart are recorded, such as atrial contraction, onset of ventricular contraction, ventricular outflow and the onset

of the period of rapid ventricular filling (37). In man the curve shows that the acceleration of venous inflow into the veins and atria during the ventricular ejection period may be sufficient to reverse the initial volume decrease caused by ventricular outflow (38).

The ballistic recoil of the heart during its contraction and the impact of the moving pulse volume against the aorta and peripheral vascular system, tend to produce movements of the whole body. These movements may be recorded in the form of a curve (ballistocardiogram) by placing a subject on a suspended bed free to move only in the longitudinal direction of the body. Starr & Rawson (39) find that curves so obtained indicate certain important mechanical features of the heart and circulation. Marked differences are present between the curves obtained from normals and from certain types of cardiac disease. The amplitude of the curve is related to stroke volume.

From experiments on dogs in complete heart block, in which atrial contraction occurs at different times in relation to ventricular systole, it appears (40) that a certain amount of blood regurgitates from the ventricle at the onset of its contraction. This is at a minimum when ventricular contraction starts shortly before the end of atrial systole, and under these circumstances the contribution of the atrium to ventricular filling is greatest. The volume pulse precedes the pressure pulse in all parts of the aorta in dogs (41). With increase in diastolic or mean blood pressure the ratio of pulse volume to pulse pressure decreases in a non-linear fashion.

Measurements of arterial and venous pressures in the pulmonary circulation of the dog with closed thorax and normal breathing (42) reveals changes in general in the same direction as in the systemic circuit, although sudden changes of considerable magnitude in the latter circuit may have little immediate influence on the pulmonary vascular pressures. It is reported (43) that occlusion of a small portion of the peripheral vascular bed in the pulmonary circulation causes an immediate rise in the pressure in the pulmonary artery, which is much greater than can be accounted for on purely mechanical grounds. The reaction is independent of the vagus and sympathetic innervation and is ascribed to an axone reflex.

In the fetus (dog and rabbit) the pressure within the right ventricle is the same or higher than in the left ventricle and both are

relatively low (44). A tendency toward the establishment of the pressure relations present in the adult animal is evident within a few hours of extra-uterine life and is complete in several days.

Considerable interest is apparent in the use of x-ray kymograph methods for recording movements of the heart borders in man and in experimental animals (45, 46). Variations in relative movements of different heart borders, related to heart rate, position of the diaphragm and blood pressure, occur in the same individual at different times (47). The two types of heart activity found in different human subjects, one showing predominant activity at the ventricular base, the other at the apex, are apparently related primarily to the position of the diaphragm (48). Direct motion picture photography of the fluoroscopic screen by the use of an ultra rapid lens is another method for studying the heart border movements. In the observations on experimental animals, iodized oil may be injected to aid in visualization of the blood movements (49).

By placing electrodes on an exposed blood vessel in the presence of a strong electromagnetic field, electrical potential differences proportional to the velocity of blood flow are induced. By suitable recording, a curve is obtained from which the velocity of blood flow may be computed. This method, developed independently by investigators in two different laboratories, has been applied in studies on the blood flow in the carotid artery of dogs (50) and in the aorta of rabbits (51). In the carotid the normal volume flow during diastole is approximately two-thirds of the systolic flow. Retrograde flow occurs during the diastolic period when aortic regurgitation is produced.

Three different types of pulsation may be recorded by means of an esophageal sound connected to a recording capsule, depending upon the position of the sound with reference to the heart and great vessels; atrial, ventricular and aortic (52).

It is well known that in the normal growing heart, the number of capillaries increases along with the increase in fiber size. This is reported to occur also when hypertrophy of the heart is induced in adult animals (guinea pigs) by prolonged physical exercise (53, 54), but increase in capillaries does not accompany the cardiac hypertrophy brought about by experimental valve lesions (rabbits) (55). Furthermore, in clinically hypertrophied human hearts, no change in the ratio of capillaries to fibers from that in the nor-

mal unhyertrophied heart is present (56); the capillaries do not multiply to accompany the increase in mass resulting from fiber enlargement. From these results it would appear that hypertrophy from physical exercise and that induced by cardiac lesions are fundamentally different processes. No detectable cardiac hypertrophy accompanies the increased heart work occurring during pregnancy in guinea pigs, dogs or cats (57).

The question as to whether or not the mammalian ventricle exhibits tonus, an alteration of length of fibers independent of intraventricular blood pressure, has long been a matter of great interest. Johnson & Katz (58) report that in the dog's heart *in situ*, driven at a constant rate by rhythmic stimulation of the right atrium, a rise of intraventricular pressure produced by occlusion of the thoracic aorta was associated, in a few instances, with decrease in ventricular volume. In most cases no evidence of tone changes appeared, the ventricular volume being determined by intraventricular pressure.

Evidence in favor of active contraction of the arteries as a factor in propulsion of blood is presented by Mougeot (59) and Marceau (60). The latter states that asynchrony of the volume and pressure pulses, normally present in an artery, disappears when atropine is applied to the wall of the vessel.

#### CARDIAC OUTPUT

Calculation of stroke volume in man from physical measurements of the vascular system, pulse pressure, wave length of arterial pulse cycle, pulse wave velocity and aortic cross section, give results for minute volume according closely with those obtained by gas methods (61). Changes in stroke volume of a transitory nature, too brief to be determined by gas methods, are revealed. A simplified gas-method procedure, in which it is only necessary to determine oxygen absorption and to make two analyses of expired air in a single rebreathing process, is also described (62).

The postural changes in minute volume in man reported in recent years, a decrease in standing as compared with the recumbent position, appears to be well established (63). The change in output also occurs where the postural change is made by means of a tilting table (64). The decrease on standing is ascribed to diminished venous return to the heart (65), since compression of the lower limbs reduces the effects of change of posture. From studies

reported in man, it appears that the minute volume output is unchanged in baths at body temperature (66), on exposure to moderately high atmospheric pressures (two to three atmospheres) (67) and in arterial hypertension (68). It is increased during early periods of exposure to high altitudes (69), a condition which also increases venous pressure (70, 71), during the initial periods of a hot bath (66), in anaemia (72), during attacks of angina pectoris (73), on exposure to a pressure above five atmospheres (67) and following the intravenous injection of saline or glucose solutions in amounts equivalent to those used therapeutically (74). The minute volume output is decreased in advanced age (75), on holding the breath, especially in the inspiratory position (76) and in cardiac decompensation (determined by the direct Fick method in six clinical cases) (77). Inflation of the lungs, a procedure carried out in many surgical operations, reduces heart output in dogs proportionately to the pressure employed (78). Raising the body temperature of rabbits by exposure to heat decreases the volume output (79) and a similar exposure in man causes instability of the cardiovascular system as indicated by increased change of output with change of posture (80). Experimental pneumothorax, at least in the early stages, reduces venous return to the heart (81).

From experiments made on intact dogs in which the total output was determined by the Fick method, and the distribution of blood estimated by oxygen consumption in different regions (82), it is reported that the flow to the head and upper extremities and through the portal system account for more than half the total. Oxygen consumption is greatest in the portal system. On increasing the arterial resistance in the heart-lung preparation of the dog, the minute volume output being maintained constant, it is found (83) that the diastolic volume of the left ventricle increases much more than when the same increase of work is brought about by augmentation of minute volume with constant resistance. The increased diastolic and stroke volume, occurring in expiration in the dog with normal breathing, is ascribed to the sudden collapse of the atrium distended by blood flow during the inspiratory phase (84).

Following exercise on a bicycle ergometer by human subjects, a period following the exercise during which the cardiac output is below normal is reported (85), while following static work (supporting a weight) it is found that the increase in output is main-

tained at a time when heart rate and blood pressure have fallen to normal (86). The physically trained individual is reported to respond to exercise with greater oxygen absorption and utilization and less increase in minute volume output than the untrained subject (87, 88). Changes in blood pressures, pulse velocities and elastic relations, in the presence of slight to large intrathoracic pressure changes induced voluntarily in man (Valsalva research), too extensive and detailed to justify a brief review, are reported by Wezler & Knabel (89).

The general method of estimating functional capacity of the cardiovascular system, by determination of oxygen absorption during a period of physical exercise and the repayment of the "oxygen debt" subsequent to the work, forms the subject of several reports. A critical study of the whole subject, and a consideration of the various factors that must be taken into account in cardio-respiratory tests, appears in the paper of Zaeper & Böhme (90). The rate of oxygen absorption during the work (91), the time following exercise for the resting rate of oxygen absorption to appear (92) and the change of oxygen absorption in passing from a lower to a higher grade of work (93) are regarded as the important features by different investigators.

#### CIRCULATION VELOCITY

Wide variations are reported in the circulation rates in different animals, estimated by the injection of radioactive material at one point in the circulation and the determination of its arrival at another point by an ionization chamber (94). The number of heart beats per circulation time is however fairly constant in different species. The rapidity of blood flow in children (calcium chloride method) is nearly twice that in adults (95). It is increased in hyperthyroidism and decreased in cardiac decompensation to a degree proportionate to that of the failure (96, 97). In 76 percent of 40 cardiac patients showing lengthened circulation time, there were other evidences of circulatory insufficiency (98). Hot baths are reported to increase, cold baths to decrease the velocity of the circulation (99). A method proposed for the determination of fractional and total circulation time, consists in the combined use of ether (vein to lungs), dicholin (vein, pulmonary circuit, left heart and systemic capillaries) and fluorescein (vein to vein; total circulation time). The average normal figures in man for these different periods are 5.6, 10.9 and 28.0 seconds respectively (100).

## HEART SOUNDS

It is stated that in man the first sound is maximum when ventricular systole starts during the period of maximum ventricular inflow as the result of atrial contraction (101). The first sound recorded from the surface of the exposed dog's ventricle is unchanged, so long as intraventricular pressure is unchanged, whether the region from which the sound is recorded is contracting normally or passively stretching due to ischaemia from coronary occlusion (102). The importance of the muscle component in the first sound is minimized in both of these reports. Analysis of the wave length and amplitude of vibrations of the first sound leads to its division into two (103), three (104) or four (105) parts related to mechanical events occurring during ventricular systole. The rapid outflow of intramural blood from the coronary system during the isometric period of ventricular contraction is suggested as a component in the first sound (106). When the aorta in the experimental animal is replaced by a non-elastic tube, the second sound is reported to disappear; to reappear when the rigid tube is replaced by an elastic rubber tube (107).

The second fetal heart sound in man is nearly as long as the first and in general shows larger amplitude of vibrations (108).

## ELECTRICAL PHENOMENA

Difference of opinion is still evident with reference to the disposition of the electric charges in the heart during activity. On the one hand the classical theory of "negativity" is supported (109), an attempt is made to express action potentials in accord with this theory by an analysis of local tissue circuits and resistances (110), and monophasic injury potentials continue to be interpreted as indicative of the electronegative change associated with activity in a normal region of cardiac muscle (111). On the other hand, other workers proceed to analyze physiological potentials on the basis of the "dipole" theory and find excellent agreement between theoretical and recorded curves (112) or to explore electrical fields around active heart muscle and to interpret them in the light of polar orientation of charges (113, 114). From experiments in which myocardiograms, aortic pressure and monophasic potentials developing between an uninjured and injured surface on the dog's ventricle are simultaneously recorded, the conclusion is derived that electrical activity precedes mechanical activity by 0.02 to



0.03 sec. (115). Complete dissociation of electrical and mechanical activity is reported in the frog's heart poisoned with formaldehyde. Electrical response may occur for as long as an hour after the heart is completely immobilized (116).

#### COURSE OF IMPULSE IN THE VENTRICLE

From high speed cinematographic observations on the frog's ventricle, the contraction appears to start normally at the base and proceeds, as a more or less discontinuous wave, to the apex (117). The normal base-apex interval is 0.06 sec. increasing to 0.14 sec. on cooling. The base remains in contraction longer than the apex. Synchronized electrocardiographic records indicate a close connection between the duration of the contractile process and the T wave.

The comparative time of appearance of action potentials at different points on the surface of the dog's ventricle is the subject of several reports. Employing monophasic potential changes derived from bipolar leads, one from an injured the other from an uninjured spot, and comparing the time of onset of the electrical monophasic curve with a standard electrocardiogram (118) led to the conclusion that all surface spots are involved within 5 to 8 msec., with the exception of the central anterior region overlying the interventricular septum which usually shows activation 10 to 17 msec. earlier. Considerably longer intervals between the involvement of different areas are reported from experiments in which the usual type of unipolar lead was employed, one electrode on the heart, the other on a distant point (leg) (119). In these experiments the animal was enclosed in a warm moist chamber to prevent cooling and drying of the exposed ventricles. In general, all surface points show an electric response within an interval of 0.0138 sec. Differences of the same order were found in experiments in which the potential changes were led to suitable amplifiers by means of the "differential" electrode (120). A small strand of yarn connecting two electrodes makes contact at its mid region to a small area of surface of the ventricle. This method has the advantage of freedom from any appreciable effect of potential changes other than those developing under the electrode or in the immediately contiguous area. Most surface points, with the exception of the surfaces over the anterior and posterior interventricular septa which are involved early, and of points on the surface of the

conus of the pulmonary artery which show late involvement, develop potential changes within 0.01 sec. of one another. All of the experiments described above are of interest in connection with the newer anatomical observation that the Purkinje system, formerly believed to end in an endocardial network, sends numerous ramifications through the ventricle to the epicardial surface.

The relation of impulse conduction to the Purkinje supply of the superficial bulbo-spiral muscle band in the dog's heart is apparent from experiments in which two electrode contacts were made with the bundle and the structure stimulated at its two ends (121). When the stimulus was applied at the apical end the electrical response resembles the normal, a result that would be expected from the Purkinje supply.

#### ELECTROCARDIOGRAPHY

On the basis of physical experiments in which the potential distribution due to an electrical dipole in various shaped fields is determined and compared with potentials produced by the heart, Katz (122) comes to the conclusion that the electrocardiogram is chiefly a record of potentials from regions of the heart in contact with good electrical conductors. The Einthoven analysis of the electrocardiogram is criticized by Fröhlich (123) on the basis that the method is only approximately true for electrical fields within limiting boundaries. It is reported that in nearly 75 percent of electrocardiograms in man the R peaks are out of phase, and determination of the electrical angle by the usual method from such curves is without significance (124, 125). The isoelectric line is reported to vary in the dog in an inconstant manner with respiration (126).

Widespread interest in unipolar chest leads is apparent from the large number of publications, only a few of which can be quoted here. Marked differences in the curve recorded result from different locations of the chest contact (127, 128) and to a less extent from the distal or "indifferent" electrode (129). In efforts to reduce or nullify the effect of potential changes in the heart on the indifferent electrode, subjects are partially (130) or completely (131) immersed in a bath or lie in contact with a large copper screen padded with gauze (132). The bath or wire mesh serves as the indifferent lead.

An interesting trend in electrocardiographic research in recent years is the combination of two or more electrocardiographic leads

into some sort of composite curve. This combination may be made by proper connections to a cathode ray tube (133, 134) or by the use of a special galvanometer with moving parts connected to the three standard leads mechanically combined to form a single curve (135). The curves so obtained are the same as the Lissajous figures well known to the electrical engineer and have a definite mathematical relation to the lead potentials from which they arise. The curves obtained are designated as vector-cardiograms, monocardiograms and triograms by different workers. From mathematical considerations and the construction of the indicated electrical circuits, the three leads may be reduced to two and the same results obtained. Two Lissajous figures may be recorded simultaneously, one from the frontal, the other from the sagittal plane of the body and combined stereoptically to form a single figure (136, 137). It is also possible to plot the absolute magnitude of vectors drawn from the null point of the Lissajous figures to its margins for various points in the cardiac cycle, and to obtain a curve known as the absolute cardiogram (134). A curve of this type can also be recorded directly by the use of proper electrical circuits interposed between the leads and recording apparatus, and this can also be done in two planes of the body. Aside from their theoretical interest, the determination of the clinical value of procedures of this type awaits the collection and interpretation of a large amount of data from normals and subjects with heart disease.

Of the very large number of general electrocardiographic observations that have appeared during the period this review covers, only relatively few can be considered here. Variations of the electrocardiogram in different individuals at different times (138), with change of posture (ascribed to differences in contact of the heart with the surrounding conducting tissue) (139), following hemorrhage (140), following reduction of alkali reserve (141), importance of angle between QRS and T vectors (142), length of cycle in hypertension (143), relation of length of cycle to QT interval (144) and the effect of a negative T wave (145), importance of size of ventricular complexes (146) and changes in length of cycle in nutritional states (147), may be mentioned. It is generally agreed that lengthening of the QT interval occurs in hypocalcemia both clinically and experimentally (148, 149), although the length of mechanical systole appears unaltered (150). Hypercalcemia is associated with shortening of the QT interval (151). Depression of

ST segment and flattening or inversion of the T waves is described in hypoglycemic shock (152) and in diabetic edema (153). Similar changes follow the injection of epinephrine in the dog (154) and in the cat (155). The ST segment change may be sufficient to produce a monophasic curve. Changes in level of the ST segment in the dog's electrocardiogram may persist for as long as an hour following the passage of an electrical current through the animal (156). The more important effects of exposure to low oxygen are changes in the ST segment and reversal of T waves (157, 158, 159). Similar changes occur in carbon monoxide poisoning (160).

Electrocardiograms obtained from human fetuses of one to two months of age resemble the electrocardiograms of infants born at term (161). Electrocardiograms from premature infants and infants at term differ from adults mainly in shorter intervals, variations of T complexes and tendency toward right axis deviation (162, 163, 164, 165). Precordial leads in children are characterized by variability in the T complexes (166, 167). The duration of "electrical systole" (QT interval) increases in the heart-lung preparation of dogs with increased heart work (168). Increase in the T wave in physical exercise in man (169) and ST segment depression following exercise (170) are described. In electrocardiograms recorded during and after clinical death, almost all types of pathological electrocardiographic changes are encountered. Some cardiac activity may persist for as long as an hour after clinical death (171).

Visualization and examination of electrocardiograms during their recording may be made by the use of a rotating belt containing phosphorescent material (172).

#### PREMATURE ECTOPIC BEATS AND BUNDLE BRANCH BLOCK

Interest continues in the question as to whether in left ventricular extrasystoles and in right bundle branch block the electrocardiogram shows left axis deviation (positive  $R_1$ , negative  $R_3$ ), the "classical" interpretation, or whether the reverse relation is present ("newer" interpretations). In experimental ventricular ectopic beats in the dog and monkey, it is found that the transition from one type of electrocardiogram to the other occurs over a boundary which does not conform to the ventricular septum or other anatomical landmark, and furthermore the position of the transition line changes with change of the position of the heart

(173). The importance of the position of the heart with reference to the results obtained is emphasized in a recent report concerning stimulation of the exposed human heart in a subject whose normal electrocardiogram showed right axis deviation. The electrocardiograms obtained during stimulation of points on the right or left ventricle accorded in general with the classical interpretation and it is concluded that the newer terminology applies only in the presence of a normal electrical axis (174). The electrocardiograms obtained in ectopic beats in the dog's heart are the same whether they result from stimulation of the endocardial surface or from corresponding points on the epicardial surface (175).

One of the difficulties in deciding as to the electrocardiographic interpretation in bundle branch block is the detailed histological examination demanded for accurate location of lesions. It is stated that careful examination necessitates the study of from four to ten thousand serial sections in a single case (176). Current reports in which careful histological studies were made favor in general the new terminology (176, 177, 178, 179).

Asynchronism in contraction of the two ventricles in dogs occurs when one branch of the His bundle is cut, the affected ventricle following the ventricle with intact branch by 0.04 to 0.06 sec. (180, 181). A definite delay in onset of the pulsation in the carotid artery in man is reported in cases of left branch block, not present in block of the right branch of the bundle (179).

#### EXTRINSIC CARDIAC NERVES

In experiments on stimulation of the peripheral vagus in the tortoise with single induction shocks it was found that two refractory periods follow the application of a single stimulus, during both of which a second stimulus produces no summation of effect. The first phase comprises a very short interval following the stimulus. The second refractory phase begins about two seconds after the stimulus and lasts for about six seconds, and is ascribed to the effect of decomposition products of acetylcholine (choline) set free by the first stimulus (182). Periods of sudden or gradual return to normal or supranormal rate during otherwise gradual recovery from vagus inhibition in frogs', rabbits' and cats' heart *in situ* are to be ascribed in part to simultaneous stimulation of accelerator fibers or to release of epinephrine from the adrenal glands. Such changes may persist however after elimination of all vasosensory

zones, extirpation of the stellate ganglia, exclusion of the adrenals and even after spinal cord extirpation. Their cause is not clear. The appearance of second periods of slowing, following partial recovery from inhibition, is explained by the action of acetylcholine present in the coronary or pulmonary venous blood returning to the right atrium (183).

The magnitude of the effect of a single stimulus applied to the vagus varies with its position in the cardiac cycle (184). This has been interpreted as indicating an intimate relation between the acetylcholine discharge and the activity of the pacemaker (184), or more recently, by antagonizing the activity process in the heart rather than its initiation (185). The first interpretation has been recently adversely criticized (186). When a vagus inhibitory effect is superposed on a simultaneous or previously initiated accelerator effect, the latter is not abolished but merely suppressed during the period of inhibitory influence. This is quite the opposite from the analogous situation in spinal cord reflexes, where an excitatory effect is completely extinguished by inhibition (187).

Experiments by Garrey and coworkers (188) show that acetylcholine is an effective test to determine whether a particular cardiac muscle is (or has been) innervated by inhibitory nerves. According to this test, the ventricle of the frog is supplied with vagus inhibitory fibers while that of the tortoise is not. The sinus and atria of both species are abundantly supplied with inhibitory fibers. Slowing of the dog's heart by vagus stimulation after interruption of the His bundle is ascribed to the passage of vagus fibers to the ventricle by some unknown path (189) or to the effect on the ventricle of "vagus substance" produced in supraventricular regions (190).

The atropinized frog's heart, showing no apparent response to vagus stimulation, nevertheless produces acetylcholine; indeed in greater amounts than unatropinized hearts (191). The dog and cat heart, sufficiently atropinized to prevent vagus action, will respond by slowing or inhibition to the injection of acetylcholine and eserine (192). It is stated that continuous intravenous infusion of acetylcholine produces sustained cardiac acceleration (193); on the other hand it is said to augment the tonus of the vagus center (rabbit) (194). After removal of both stellate ganglia, graded inhibitory effects are lost; vagus stimulation is either ineffective or produces complete cardiac inhibition (193).

Acetylcholine is reported to decrease systolic output in the isolated frog's heart, while the diastolic volume is unchanged or slightly increased. Epinephrine increases systolic output with unchanged or slightly decreased diastolic volume (195). Tone in isolated strips of the tortoise atrium is much increased by acetylcholine and by potassium excess (196). In both instances the effects are antagonized by atropine. The heart of the snail (*Aplysia fasciata*) is stopped in a condition of extreme dilatation by concentrations of acetylcholine of the order of  $10^{-7}$  in the sea water used for perfusion (197).

A definite increase in potassium in a perfusate through the vascular system of the frog occurs when the vagus is stimulated (198). Changes in calcium were insignificant. Increase in potassium or decrease in calcium in the solution perfusing the toad's heart increases vagus excitability. Increased calcium decreases the excitability (199).

An increase in heart rate on distention of the right atrium (Bainbridge reflex) can be demonstrated even though the vagi are cut (200). Animals in which the vagi are cut and carotid sinus denervated, usually show increased heart rate with increase of arterial blood pressure (201). The heart rate in man changes with change of blood pressure in the same way as in the experimental laboratory animal (202). In isolated perfusion of the carotid sinus in dogs it is found that the cardiac reflexes are more variable and less well maintained than the associated vasomotor reflexes, although the sympathetic efferent component of the carotid sinus-heart reflex can be demonstrated (203). Accelerator fibers to the heart are found in the vago-sympathetic trunks of the dog (204), although they probably have little physiological significance since emotional excitement and atropinization does not increase the heart rate above the automatic rate in dogs in which total parasympathetic sympathectomy has been performed, whether both vagi are intact or the right vagus is cut (205). Ventricular acceleration following stimulation of the stellate ganglion after section of the His bundle is reported (206). Reflex cardiac inhibition occurs in the frog when the various hollow viscera are inflated (207). In addition to cardiac slowing, heart block and ectopic impulses may result from pressure on the eye ball in man (208). By the injection of eserine in human subjects it is possible to demonstrate that vagal substance is liberated in many instances in the blood of man and



in greater concentration in the cerebrospinal fluid of dogs when this procedure is carried out (209).

Diminished heart rate response to exercise (210, 211) and to increase in skin temperature (212), and a decrease in the resting respiratory arrhythmia (213) are characteristic of increasing age in the human subject.

An increase in heart rate occurs when the epicardial surface of the heart is submitted to the action of a local anaesthetic, indicating the presence of sensory nerves which reflexly influence heart rate (214). Increase in rates of perfusion of the isolated pulmonary circuit of the dog may increase or decrease heart rate, both before and after vagotomy (215). The initial slowing of the heart rate in man following the administration of adrenalin appears to depend on the vagus, but is independent of any change in blood pressure (216).

Extensive experiments concerning the rôle of the vagi, carotid sinus and aortic nerves in the reactions to oxygen lack in the dog are reported (217). The slowing of the heart in moderate oxygen reduction is attributed to a central vagus effect. Reflex accelerator stimulation appears to play little rôle in the reactions. An acceleration of heart rate is an early feature following exposure of man to gradually decreasing oxygen pressure (218). On further decrease of pressure collapse occurs earlier in those subjects showing a smaller initial acceleration (219).

In rats and kittens, submitted to low surrounding temperature, the heart rate falls in a linear relation to the fall of body temperature. A heart rate one-third the normal may result. The effects are believed to be central (220).

In experiments on fetal sheep of different ages (221) it is found that vagus control over the heart is very slight or absent in the 88 day fetus, but is fully developed by the 119th day of fetal life.

#### METABOLISM

Administration of epinephrine combined with physical work causes a rapid lowering of the glycogen of the dog's heart. When it is exhausted, heart failure supervenes. If the depletion is stopped short of failure no restoration of glycogen occurs if the blood lactate and glucose are low; glycogenesis is absent or minimal. The addition of lactic acid is without effect, but if glucose is administered there is rapid restoration of the muscle glycogen. The

source of glycogen in the dog's heart is thus glucose (222). The glycogen cycle in mammalian heart muscle appears to be qualitatively the same as in skeletal muscle but of less magnitude, due to lower enzyme content (223). Glycogen cannot be the sole source of energy for the frog's heart since it continues to beat and forms ammonia and urea for a long time after poisoning with iodoacetic acid (224).

Previous work on the mammalian heart has shown that the total consumption of oxygen cannot be accounted for on the basis of carbohydrate oxidation alone. Experiments on heart-lung preparations of dogs indicate that fat is also oxidized, but the results are not regarded as entirely conclusive (225).

The presence of amino acids increases the lactic acid production in the frog's as well as in the rabbit's heart, whether due to transformation of the amino acids into lactic acid or to stimulation of glycogenolysis is not clear. No similar effect occurs in skeletal or smooth muscle (226). Heart muscle from dogs in which both pancreas and hypophysis have been removed manifests normal carbohydrate metabolism (227), in contrast to the loss of carbohydrate utilization following removal of the pancreas alone. The relation of diastolic fiber length to energy release by the heart is an important (228) but not the sole factor, since different factors causing the same diastolic volume are not necessarily associated with the same amount of work, and various drugs may increase oxygen consumption without altering diastolic volume (229). Alterations of the hydrogen-ion concentration of the blood in the perfused heart-lung preparation may also alter oxygen consumption to a greater degree than can be accounted for by changes in frequency or heart work produced by the pH change (230). The extrinsic cardiac nerves appear also to exert an important effect. In a heart-lung preparation in which the nerve supply is preserved, oxygen consumption at all work levels is less; the heart is more efficient (231). In the failing heart also the normal close relation between diastolic fiber length and oxygen consumption no longer exists, the rate of oxygen consumption decreases as the ventricles become more dilated (232, 233). The mechanical efficiency is however not reduced until failure is nearly complete, and cardiac failure should be regarded as a decrease of ability to release energy rather than a decrease of mechanical efficiency (233).

The left ventricle has a greater creatine content than the right,

in the dog (234), and in man, except in the fetus and new born where the reverse appears to be true (235). In the new born the total creatine content of the heart is low, reaching adult value a few months after birth and well before the saturation level is reached in skeletal muscle (234). No loss of creatine from the artificially perfused rabbit's heart occurs (236), although marked loss in the dog's heart is reported in a region of ventricle perfused through its coronary system (234). Creatine is formed from arginine in the isolated rabbit's heart (236). The creatine content of the hearts of patients dying with heart failure is in general lower than in normal human hearts, although marked variations are found in individual cases (235).

The histamine content of the specific musculature is reported to be much greater than the myocardium, and is greater in the right than in the left ventricle (237). Recent observations fail to confirm older observations that histamine is produced in the isolated dog's heart (238).

The isolated atrium of the rabbit, in buffered solutions and deprived of oxygen, shows rapid failure, accompanied by a marked reduction of its glycogen, phosphagen, and adenosinetriphosphate. During recovery in oxygenated solution the phosphagen is restored and the improvement in mechanical response appears to parallel the phosphagen content (239). Similar losses in energy yielding materials are reported to occur in asphyxial arrest of the isolated cat's heart (240). The survival time of the dog's heart under similar conditions is prolonged when an adequate supply of glucose is present (241). After ligation of a coronary artery, the coronary venous blood shows a rapid rise in lactic acid content and increase in hydrogen ion concentration (242). A loss of twenty to fifty percent of the adenylypyrophosphoric acid occurs in guinea pigs' hearts in experimental thyrotoxicosis (243).

Human hearts, normal and diseased, contain about 64 milligrams of phosphatide nitrogen per gram of muscle (244). The epinephrine content of guinea pig hearts is reported as 0.1 to 0.2 gamma per gram of muscle. The atrial and ventricular muscle content is the same (245).

From experiments on the embryonic heart of the marine minnow, exposed to carbon monoxide poisoning at different light intensities and to cyanide poisoning, it is concluded that Warburg's respiratory enzyme is in part, but not entirely, responsible for gas

metabolism in this species (246, 247). When the tortoise heart is bathed with a solution in which the water content contains 50 to 96 percent of "heavy water", the amplitude and rate of contractions is reduced and early arrest occurs (248).

Degenerative changes in the myocardium occur following unilateral vagus section in rats, guinea pigs and rabbits (249) and following prolonged vagus stimulation in the dog (250). The effects of the latter are prevented by atropin and accentuated by eserine.

#### VITAMIN DEFICIENCIES

Vitamin-B<sub>1</sub> deficiency in rats causes bradycardia, not of vagus origin, and depression of the T wave and the ST segment of the electrocardiogram (251). It is not clear whether or not the bradycardia is due to certain metabolites produced in excess in this condition (lactic and pyruvic acids and  $\alpha$ -ketoglutaric acid) (252). Synthetic vitamin administration in normal dogs is reported to produce, on the other hand, a marked and sustained bradycardia (253). The addition of vitamin C to the perfusion solution of the isolated frog's heart increases the extent of contraction, especially when added after perfusion has been carried out for some time (254). Acute vitamin-C deficiency in the guinea pig is reported to be associated with proliferative lesions along the margins of the heart valves (255). No changes in the electrocardiograms of normal children receiving therapeutic doses of vitamin D over a long period of time occur, contrary to the results of previous workers (256).

#### RESUSCITATION

Of 125 clinical cases in which the heart had stopped as a result of anaesthesia or other factors, restoration of normal beat and recovery was obtained in 5 by the intraventricular injection of 1 mg. of epinephrine (257). Passage of a direct current through the hearts of rats and rabbits following cardiac arrest or during ventricular fibrillation is reported to restore a normal heart beat if the heart is connected to the anode. Connecting the heart to the cathode pole of the direct current induces or increases fibrillation (258). The intravenous injection of procaine hydrochloride followed by an electrical shock through electrodes placed on the heart is effective in restoring a normal rhythm to the fibrillating dog's heart (259).

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PHYSIOLOGICAL LABORATORY  
UNIVERSITY OF WISCONSIN  
MADISON, WISCONSIN

## ELECTRICAL PHENOMENA OF THE BRAIN AND SPINAL CORD\*

BY HALLOWELL DAVIS

*Department of Physiology, Harvard Medical School  
Boston, Massachusetts*

The present review aims to cover the literature of the electrophysiology of the brain and spinal cord from early in 1937 until September 1938. The review by Jasper (70) and the monograph by Kornmueller (77) define the beginning of the period. With a few exceptions, articles cited by either author are not included. No attempt has been made to close the chapter at an exact date. Some articles still in press are cited and others already published may have been omitted entirely.

### METHODS

The rapid recent advances in the study of the electrical phenomena of the brain are directly based on the steady improvement in recording technique. Ink-writing electroencephalographs are not new (118), but have been steadily improved (93) until we now have instruments of sufficiently high natural period and uniform frequency characteristics to allow faithful recording of frequencies up to fifty cycles or more. With an ink-writer it is practical to take multiple records over long periods of time and to repeat observations freely (56) without the drawbacks of expense and delay involved in photographic recording.

A technical advance which may prove of considerable utility is the photo-electric analyzer (53, 60, 61) developed for analysis of electroencephalograms. The analysis gives the "frequency spectrum" of the record, *i.e.*, the distribution of energy as a function of frequency. The frequency spectrum is a form of objective description which should prove a useful supplement to the usual method (cf. 35) of visual recognition of particular patterns. It yields the same type of information as a Fourier analysis (101, 102) and should prove both quicker and more objective than the current methods of measuring and counting individual waves. A compromise method, very useful in the study of particular fre-

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quency bands, is based on broadly tuned electrical filters included in the original recording system (32).

Microelectrodes have been successfully applied to the brain of the living animal (50, 100, 46), and offer great promise of identification of particular electrical discharges with particular types of cell, and possibly the differentiation of the activity of dendrite cell-body, and axone.

#### ANIMAL EXPERIMENTATION

*Spinal cord.*—Electrical potentials of the spinal cord may be led off directly from its dorsal surface (68) and also from the spinal roots (3, 17, 18). From the dorsal surface both positive and negative waves are obtained. Hughes, McCouch & Stewart (68) attribute part of the electrical complex to internuncial neurons, and they emphasize the parallelism between reflex inhibition or facilitation and modifications of the "internuncial complex."

Electrodes placed on the spinal roots near the cord yield slow electrical waves which are not generated locally in the spinal roots but spread outward by "electrotonus" from the gray matter within the cord. The electrotonic waves evoked by sensory stimulation of peripheral nerves invariably show increased negativity of the lead nearest to the cord (3, 17). There are systematic differences between waves from posterior and from anterior roots, but it is certain that the slow electrical changes in the anterior root are closely associated with the initiation of impulses in the anterior horn cells. The various investigators offer interesting but somewhat divergent theoretical interpretations of the origin and significance of the potential waves.

The general character of the electrical activity of the gray matter of the central nervous system seems to be much the same wherever it is studied, whether in the spinal cord, in the medulla (16), in the superior colliculus or lateral geniculate body (128, 129, 130), in the thalamus (116, 44, 45), in the hypothalamus (62), in the cerebellum (116, 34a), in the hippocampus (100), or in the cerebral cortex. All investigators describe slow, often rhythmic, waves usually between 0.05 sec. and 0.2 sec. in duration. Faster and sometimes still slower components are usually present also. Some activity is usually "spontaneous," in that it occurs without special sensory stimulation and persists (although perhaps less vigorously) after cutting some or all afferent pathways. The "spontaneous"

activity appears to be less prominent in the spinal cord than in the higher centers.

*Localization of response and functional interconnections.*—Electrodes applied to the cerebral cortex detect electrical changes occurring in the immediate neighborhood of the electrodes and are very slightly affected (if at all) by electrical activity in more remote regions, even by regions lying between the points of application of the electrodes (78). This principle applies both to "bipolar" leads (both electrodes on cortex) and to "unipolar" leads (one lead on cortex, the other on remote bone or muscle). Although the evidence is less complete for the case of intracerebral (*e.g.*, thalamic) electrodes and for electrodes applied to the outside of the skull, it seems safe to infer that the principle of local origin near an electrode is generally valid, at least as a first approximation. Small movements of electrodes may be quite critical in determining the detection or failure to detect electric responses. Therefore, inferences may legitimately be drawn as to localization of response and, in turn, as to functional interconnections of particular anatomical regions.

The representation of tactile sensibility in the cortex of the monkey has been accurately determined by the localized electric responses to peripheral stimulation (96, 94). A cortical center for taste in the rabbit in the region in the center for mastication is described (49). The portion of the cortex in the rabbit which reacts to stimulation of the optic nerve has been delimited (98), and there is some evidence of specific representation of particular regions of the retina (5). Excitation of the cerebellum apparently exerts a stimulating influence on the cerebral motor cortex (126). Stimulation of the central end of the vagus nerve increases the electrical potentials of the orbital surface of the frontal lobe (2). Definite functional boundaries appear in the sensorimotor cortex of the monkey (40, 42, 43, 48). For example (42), local application of strychnine to a small portion of the "post-central arm area 1 or 2" causes the appearance of typical excitatory "spikes" in arm areas 1, 2, and 5. But, whereas strychninization in arm area 2 "fires" arm area 4, strychnine in arm area 1 decreases the electrical activity of area 4, and in neither case is the leg or face area "fired." In a series of brilliant experiments (40, 45) the functional interrelationships of the motor cortex, the nucleus caudatus and the optic thalamus have been mapped.



*Analysis of cortical activity.*—The electrical response of the optic cortex of the cat to single electrical stimuli applied to the optic nerve has been analyzed in some detail (4, 5, 7, 8, 14, 98) by means of needle electrodes inserted at various depths into the cortex. The response consists of three overlapping series of potential waves differing in their time-relations (14). The first series consists of three or more rapid waves of duration similar to axone spikes. The first rapid wave is definitely assigned to the afferent radiations, and the second to cortical neurons corresponding to the intercalary neurons of the spinal cord. The third rapid wave represents another type of neuron, apparently efferent from the cortex to the superior colliculus. The second, slower, series consists of two or three waves, the first surface-positive, the second surface-negative. Each wave has a duration of 5 to 10 msec. The third and still slower series has the dimensions of the spontaneous ("alpha") waves, about 100 msec. for each wave, and presumably involves the activation of the "alpha" mechanism by the stimulus. The second series is an immediate and relatively localized response, more or less specific in nature. Local application of strychnine serves to differentiate the second from the third series. The second series is increased in amplitude and, with heavier doses, becomes repetitive and finally appears spontaneously [the "firing" of Dusser de Baronne (42)]. The second series must not be confused with the spontaneous "beta" activity of the cortex, in spite of superficial resemblances in the duration of its waves, for the "beta" waves are unaffected by strychnine and, unlike spontaneous strychnine-waves, are suppressed by deep anesthesia. The slowest series of waves is not enhanced by strychnine, but, instead, under heavy doses it is depressed to extinction. This slowest sequence persists under relatively deep anesthesia. The differential behavior of the two sequences indicates that they occupy separate cortical structures. There is no evidence of transition from one form of wave to the other.

The foregoing analysis by Bishop and his collaborators of the electrical response to afferent stimulation is the most complete yet available. The waves described by other investigators (cf. 20) probably correspond to one or both sequences of slow waves, but, without further experiments, it is dangerous to attempt to identify in terms of Bishop's analysis either the "secondary discharge" under barbiturate anesthetics (34, 19, 21), the responses utilized by Marshall (96) to map the sensory cortex, or the widespread "K"

phenomenon of Loomis, Harvey & Hobart (93) appearing from the brain of sleeping humans.

Dusser de Barenne & McCulloch (46) give important additional analysis of the cortical electrogram, based on the methods of thermolaminar coagulation, microelectrodes, and local strychnine poisoning. The three outer layers of a zone in the motor cortex of a monkey were killed by thermocoagulation. Six weeks later when the killed layers had been completely absorbed, the animal was tested electrically under dial anesthesia. No difference could be detected in the (bipolar) electrical records from "reduced" and from normal cortex. The differences previously (41) obtained immediately following coagulation are explained as due to, first, the development of acidity in the coagulated tissue and, secondly, the presence of the coagulated layers, which modifies the local electrical circuits.

Study of strychnine "spikes" by means of a microelectrode inserted to various depths (46) shows that the spikes generated in the superficial layers are surface-negative relative to a distant electrode. When all layers are affected by the poison, a preliminary surface-positive phase is recorded by an electrode at the surface, but when the microelectrode is inserted to the pyramidal cell layer only negative spikes are obtained. Thermolaminar coagulation of the three outer layers abolishes the surface-negative phase recorded by a surface electrode and leaves the preliminary surface-positive spike. This surface-positive spike is identified with the negative spike recorded by a microelectrode deep in the cortex. Dusser de Barenne & McCulloch interpret the fully developed strychnine sequence as a wave of organized activity originating in the deeper layers, rising up to involve the surface layers, and then returning again to the depths.

*Effects of sensory stimulation and of deafferentation on the "spontaneous" activity of the cortex.*—The electrical record from the brain of an unanesthetized rabbit, like the record obtained from the intact human head, varies from moment to moment. Slow sequences of rhythmic waves, 7 or 8 per sec. in the rabbit, 8 to 13.5 per sec. in the human, alternate with periods in which only smaller, faster "beta" waves are present (49). The oscillogram becomes more regular and low-frequency waves predominate as the animal passes from the waking to the sleeping state. In the awakened animal, sensory stimulation causes suppression of the "alpha"

waves (as in the human) and increase in the amplitude and frequency of the "beta" waves. [The "beta" reaction may sometimes be an increase, sometimes a decrease in amplitude (6).] The cortical sensory reaction is greatest in the region of the projection of the excited organ, whether for vision, smell, hearing, taste, or touch. An intense stimulus causes a more widespread reaction which may involve the entire cortex. Furthermore, in the waking animal, the sensory reaction is associated with visible manifestations of attention, interest, or emotion, such as fear (49). The spontaneous, more or less rhythmic, activity of the cortex is reduced by transection of the brain-stem (20, 22, 87). In general, the higher the section, the less spontaneous activity remains. The greatest reduction of activity follows interruption of the thalamo-cortical pathways. The dependence of cortical activity upon subcortical connections is more complete under pentobarbital anesthesia than in the unanesthetized animal. Bremer (22) suggests that incoming sensory impulses maintain a "cortical tone" which is expressed by the electrical activity (cf. also 35, 89). This view differs from the earlier suggestion of Bishop (13) that the usual rhythmic activity represents a "reverberation" of activity back and forth in closed circuits between cortex and thalamus. Both concepts are plausible, and they need not be mutually exclusive.

*Modifications produced by anesthetics and other agents.*—It is impossible to enter into detail concerning modifications of electrical activity by anesthesia and other agents. Ether reduces the sensory reactions of the cortex. Drugs of the barbituric acid series produce a picture broadly resembling that of normal sleep (or the deafferented cortex) with persistence and even amplification of the primary reaction to stimuli, although the more widespread after-discharge or secondary reaction may be abolished (19, 21, 14). The spontaneous electrical activity of the cortex often becomes simpler and more clearly rhythmic and periodic, and the patterns recorded from different regions resemble one another more and more closely (36, 37). Dial anesthesia prolongs the refractory period of the sensory systems (95). Fall in blood pressure induces changes which closely resemble those caused by increasing the depth of anesthesia (9, 2). There are important correlations between electrical activity, electrical excitability, steady-state potential gradients, and the pH of cortical tissue. In particular, increase of pH above 7.3 augments both activity and excitability, and decrease of pH di-

minishes both. Conversely, the cortical after-discharge which follows stimulation reduces the pH of the tissue in which it occurs (47). Potassium, acetylcholine and eserine, in small doses, increase electrical activity, while calcium, atropine and large doses of potassium depress or modify it (17, 18, 97, 89). In man, the frequency of the "alpha" rhythm may be somewhat increased, at least temporarily, by thyroid medication (109; cf. also, 63), but the observations of Ross & Schwab (106) on a group of patients with thyroid dysfunction raise doubts as to the real significance of the correlation ( $r = 0.688$ ) which Ross & Schwab themselves report between "alpha" frequency and basal metabolic rate.

#### THE HUMAN ELECTROENCEPHALOGRAM

Many studies have been devoted to the human electroencephalogram, that is, the electrical activity of the brain as recorded through the intact skull and scalp. The problem is still in the descriptive stage. Current definitions, classifications of various types of waves, and available quantitative data have been summarized elsewhere (29).

The tendency to individuality and to reproducibility of pattern under standard conditions has been confirmed implicitly by numerous investigators and explicitly by others (107, 108, 88, 93). As a single index Liberson (88) chooses the median amplitude of all waves of more than 0.05 sec. duration, and he finds that the variation between individuals is far greater than the variations of a single subject. The median amplitude for 75 subjects is about 15 microvolts.

Rubin (107) determined the mean values of the "per cent time alpha" and also the mean amplitude and mean frequency of the "alpha" waves in sixteen normal and in twenty schizophrenic individuals. The mean values for the two groups are nearly identical. An analysis of variance demonstrates that both the normals and the patients in a given experimental session show insignificant variation of "per cent time alpha," of number of "alpha" trains, and of average length of trains. The patients showed a significantly greater day-to-day variation than did the normal controls. Individuality of "alpha" rhythm is indicated by the variance of "per cent time alpha" between normal individuals, which is 122 times greater than the variability from day to day for a given normal subject.

The foregoing figures are based on records from the occipital region, where the "per cent time alpha" is most constant. The amount of "alpha" activity may be as high or, at times, higher in the frontal region than at the occiput, but in the frontal region the "alpha" activity fluctuates more widely from moment to moment and from day to day (108). Several investigators (108, 88, 72a, 105, 69) emphasize the point, which is now generally accepted, that the "alpha" rhythm may arise independently in all regions of the cortex, and that it does not depend upon a particular "focus" in the occipital region. Bursts of "alpha" activity are not necessarily simultaneous in various regions of the brain, and the "alpha" frequency may be slightly but definitely slower in the frontal than in the occipital region.

*Infancy and childhood.*—At three or four months of age, rhythmic waves with a frequency of three to four per sec. first appear over the occipital lobes. These are apparently homologous with the adult "alpha" waves which have a frequency of 10 per sec. Thereafter the waves increase gradually in frequency, in amplitude, and in length of synchronous sequences. The adult frequency is reached at eight or ten years (114, 112, 90). The increase in frequency with age can be described empirically by an exponential equation (131). Smith (113) and Lindsley (90) both describe the development of other rhythms ("beta," "delta," etc.), both faster and slower than the "alpha." Lindsley believes that the other rhythms accelerate with age like the "alpha" rhythm, while Smith believes that the changes are in amplitude and regularity rather than frequency. It is noteworthy that Smith finds that the "spindles" of sleep with a frequency of 14 per sec. appear (cf. 92) as early as the first week of post-natal life, and that the sequence of changes with the onset of sleep in infants (113) conforms quite closely to the description (32) for adults.

The possible relationship of mental age to features of the electroencephalogram, such as the frequency, the amplitude, and the prevalence of "alpha" waves, has been studied (79 to 84, 99) and certain correlations appear to be statistically significant, but no general conclusions can yet be drawn, as it appears that the type of mental deficiency and probably also the degree of accompanying structural abnormality are very significant factors.

*Sleep.*—The pattern of electrical activity changes systematically with the onset of sleep (92, 31, 32, 15, 105). With drowsiness

the "alpha" rhythm with its frequency of 10 per sec., if it is originally present in the waking record, diminishes in voltage and the intervals between trains of "alpha" waves become longer. The low-voltage intervals during which the "alpha" rhythm is absent and the "beta" waves are reduced have been correlated with the subjective experience of drowsing or "floating," *i.e.*, the loss of awareness of immediate sensory stimuli. [Cf. also a description of electrical patterns during a self-induced "trance" state (117)]. In the next stage of sleep, waves of 0.25 to 0.15 sec. duration, sometimes in regular sequences, begin to appear, particularly in the precentral region. The "beta" waves slow down progressively. A characteristic stage of moderately deep sleep shows well-marked "spindles" (*i.e.*, brief trains of about one second duration) of waves at 14 per sec. appearing on a random wavy base-line. As sleep becomes deeper the random waves increase in voltage and wavelength, until in very deep sleep they may reach several hundred microvolts in amplitude and lengths of two seconds or more. The waves with frequency of 14 per sec. are absent in this very deep stage, or their frequency has fallen to 12 or 10 per sec. The depth of sleep in terms of resistance to arousal by external stimuli corresponds closely to the potential pattern, particularly to the amplitude and duration of the slow "delta" waves. All individuals show fundamentally similar patterns of electrical activity when they are asleep.

A disturbance during sleep, notably from auditory stimulation, may partially awaken the sleeper, with return of his "alpha" waves. Sometimes it evokes large waves with a frequency of 7 per sec. More often, and particularly during the stage when the spindles with frequency of 14 per sec. are present, it initiates a large slow sequence of waves, the "K complex" (93). The "K complex" is most prominent in the precentral and least prominent in the occipital region. The scalp becomes negative, then positive, relative to the ears, and oscillations may continue rhythmically for several seconds. Quicker waves, from 15 to 8 per sec., are superimposed on the later phases of the slow waves. "K complexes" may also appear spontaneously, and their slow components merge imperceptibly into the random potential pattern of deep sleep.

*Effects of sensory stimulation.*—Many investigators, notably the Iowa group (120 to 125, 75, 76), have investigated the effects of visual, auditory and tactile stimuli on the normal waking pat-



tern of electrical activity. Visual stimuli regularly produce a "check" or "blocking" of the "alpha" rhythm, and many data summarized elsewhere (29) are now available on the latency of the reaction and its persistence after the stimulus. Most authors agree that psychological factors such as attention, interest, startle, etc., are important in determining the reaction. Auditory and occasionally tactile stimuli suffice to check the "alpha" rhythm (39). In all of these experiments the interest has centered almost exclusively on the "alpha rhythm," but in addition definite "on-effects" have been noted in response to both light and sound (27, 132, 72a). The "on-effects" are sometimes described as "shifts in the baseline," and have been attributed to movement of the subject (105),—but analogy with observations on animals and on the sleeping human indicates that they are real phenomena deserving further study.

*Relation to muscular activity.*—No change in the pattern of electrical activity has been identified as associated with voluntary muscular activity, but normal tremor movements seem to correspond closely with the frequency of potential rhythms from the precentral region (72). The major rhythm in each case is about 10 per sec. and the minor rhythm about 25 per sec. During normal sleep the tremor rhythms show changes which parallel the cortical potential rhythm, *i.e.*, slow rhythms of about 5 per sec. and bursts of waves at a frequency of 14 per sec. Sensory stimulation in the waking state may depress the tremor or cause a dissociation of the cortical from the muscular rhythm. In paralysis agitans the tremor rhythm rarely (72) if ever (111, 134) appears in the cortical record. In epileptic patients, however, the clonic muscular movements may be clearly associated with characteristic cortical "seizure waves" (72).

*Relation to autonomic activity.*—In an interesting case of voluntary control of autonomic activity [erection of hairs, dilatation of pupils, increase of heart rate (91)], the subject showed localized electrical "changes" over the premotor area which preceded and appeared to be associated with the peripheral autonomic changes. The galvanic skin reflex, although evoked by the same "startle" stimuli that are effective in checking the "alpha" rhythm, is not necessarily associated with the latter reaction. The modification of the "alpha" rhythm fades out much more rapidly with repeated stimulation than does the galvanic skin reflex (51).



*Effects of drugs, anoxia, acapnia, etc.*—The effect of drugs on the human electroencephalogram has been summarized as follows by Gibbs, Gibbs & Lennox (55): "Only those drugs which were given in sufficiently large doses to cause impairment of consciousness or involuntary muscular movements produced marked alterations in the EEG. Drugs which cause a sleeplike state altered the EEG in the same general way as natural sleep. Drugs which cause a profound abolition of consciousness produced records similar to those seen in stupor from whatever cause. Drugs which cause convulsions resulted in alterations of electrical activity similar to those which occur in the convulsions of epilepsy. The more nearly alike the clinical action of two drugs, the more nearly alike are the changes which they produce in EEG's."

The original articles must be consulted for detailed information concerning the effects of caffeine, cocaine, atropine, and morphine (10), mescaline (25), and metrazol (26, 62a). Insulin, in sufficient doses to produce severe hypoglycemia and coma, causes loss of "alpha" activity and a great increase in slow waves (64, 65, 134). The effects of reduced oxygen tension have been studied in man (86, 30) and in animals (23). The most complete description (86) states that neither moderate anoxemia nor increase in oxygen tension produces much effect, but anoxemia sufficient to produce unconsciousness is attended by a great increase in the slow cortical potentials. The frequency of the electrical waves is more delicately sensitive to alterations in the tension of carbon dioxide than to moderate changes in oxygen tension. Progressive increase in the carbon dioxide content of arterial blood causes increase in the frequency of the potential waves, and decrease of oxygen tension causes a decrease in frequency [but cf. also Bremer & Thomas (23)]. Coincidentally, there is a profound alteration of cerebral blood flow, as if to protect the brain against the effects of excessive concentration of carbon dioxide.

All of the foregoing statements as to changes in the frequency of the cortical potentials are merely descriptive of the electrical records as a whole. It is not yet clear whether the alterations represent primarily acceleration or slowing of the normal "pacemakers" or, on the other hand, increases in amplitude of pre-existing rhythms of different frequency, or even the activation of previously dormant mechanisms. As a measure of the increase in slow-wave activity, Hoagland, Cameron & Rubin (64) propose the "delta

index" and describe the changes in this index during insulin treatments of schizophrenia (64, 65, 67). It remains to be seen whether this index proves to be a generally useful method of evaluating increases of slow-wave activity.

*Interpretations.*—A few papers are primarily devoted to summarizing information. Others suggest more or less speculative interpretations of the origin and significance of various features of the electroencephalogram and of its relation to purely psychological factors (10, 11, 12, 29, 83a, 101, 104, 105, 110, 52, 119). Space does not allow exposition of their content.

*Epilepsy.*—The electroencephalogram during epileptic seizures shows distinct and characteristic potential waves, and electroencephalography is becoming of increasing clinical utility in the diagnosis of epilepsy and in evaluating the effectiveness of therapeutic measures in its treatment. Gibbs, Gibbs & Lennox (54) summarize their extensive studies as follows: "The rhythm which obtains during seizures is distinctive for the three main types [of epilepsy]: *Grand mal* has a fast, psychomotor (psychic variants), a slow, and *petit mal*, an alternating fast and slow rhythm. The exact pattern . . . tends to be characteristic for each patient . . . . Some patients have subclinical seizures which are typical short disturbances of rhythm not attended by subjective or objective evidence of a seizure. *Petit mal* may occur during sleep . . . . In some patients abnormal activity begins in one area of the cortex and spreads to involve other areas . . . . The inhalation of carbon dioxide and the administration of glucose are effective in abolishing certain abnormal rhythms . . . ." (Cf. also, 12, 56, 57, 73, 74, 86a.) Golla, Graham & Walter (58) note that in 91 out of 214 cases of epilepsy the electroencephalograms taken between seizures were definitely abnormal. The abnormal features were usually slow ("delta") waves, and they occurred most frequently in patients under forty years of age with clinical diagnoses of "idiopathic epilepsy" and histories of major fits. In most such cases the abnormality of the electroencephalogram was both persistent and localized.

*The electroencephalogram in cases of tumor and other organic lesions of the brain.*—The most significant recent advance in the clinical application of electroencephalography has been in the recognition and localization of tumors and other organic lesions. Modifications of the ("alpha") rhythm with frequency of 10 per

sec. in the presence of organic lesions have been described (85), but it now appears that the local appearance of high-voltage fast ("beta") waves or of slow ("delta") waves is far more significant (127, 24, 133, 134). Walter (127) applied Adrian's (1) method of localization and demonstrated the local origin of slow "delta" waves in regions of cortical tumor, local degeneration, or edema. The "delta" waves are similar to the slow waves found throughout the cortex during ether anesthesia and in the pathological unconsciousness due to greatly increased intracranial pressure. It is the partially degenerate cortex, not the tumor or completely degenerate cortex, which produces the "delta" waves (127, 133).

It has been pointed out (12, 64) that some schizophrenic patients show an abnormally large amount of slow-wave activity [a high "delta index" (64)] and that the index might be correlated with the clinical status of the patient. On the other hand, there is no alteration of the electroencephalogram which is characteristic of the psychoses (64, 30, 71, 134). The electroencephalogram of a psychotic patient may be perfectly normal. Nevertheless, a high percentage of psychotic patients do have abnormal features in their electroencephalograms and the suggestion is obvious that these patients may have local or general cerebral lesions, or else conditions related to epilepsy, which are at least in part responsible for their abnormal mental condition (30, 71, 57).

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DEPARTMENT OF PHYSIOLOGY  
HARVARD MEDICAL SCHOOL  
BOSTON, MASSACHUSETTS

## THE SPINAL CORD AND REFLEX ACTION\*

BY J. C. ECCLES

*Kanematsu Memorial Institute of Pathology  
Sydney Hospital, Sydney, Australia*

The center of interest in the physiology of the spinal cord and reflex action lies at present in the field of general physiology of reflex action, and this review makes no attempt to cover other investigations on the spinal cord, *e.g.*, those with an anatomical or embryological basis. As far as possible the literature has been examined to the end of July 1938.

### THE SLOW ELECTRICAL RESPONSES OF THE SPINAL CORD

Until Gasser and Graham (23) in 1933 recorded the electrical responses of the spinal cord, all systematic investigations of reflex action had been confined to experiments in which nerve impulses were fired into the central nervous system via the afferent or efferent nerve fibers, and the discharge of impulses from the central nervous system was recorded either directly from the efferent nerves or indirectly from the responses of effector organs—muscles or glands. All information on the central processes was derived by analysis of the recorded outputs of impulses in response to what have been regarded as known and pre-determined inputs (10). In the original investigations of Gasser & Graham (see also 30, 31, 55, 32, 22), the electrical leads were placed on the spinal cord, usually on the dorsal surface, and hence recorded in a relatively non-selective and random manner from the aggregate of nerve cells in the grey matter. However, these investigations have strongly suggested that the slow waves of the electrical response are produced in the internuncial neurones (hence their name—intermediary potentials) and that, as would be expected, there is a close relationship between these intermediary potentials and the reflex responses of the spinal cord. Thus Gasser (22) concludes that "it is possible, through observation of the potentials alone, to predict the character of the reflex which will be produced by the testing volley,"—that is whether it will be facilitated or inhibited. A preponderatingly negative intermediary potential is indicative of facilitation, a preponderatingly positive of inhibition.

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This conclusion suggests a similarity between the intermediary potentials and the slow negative and positive potentials produced by ganglion cells; and it has been argued (15, pp. 384-7) that all the apparent differences, such as the interaction of the potentials produced by successive nerve volleys, are explicable by the known complexity of the interconnections of the internuncial neurones (40).

An important advance was made when Barron & Matthews (4, 5, 6) employed the technique of selective leading from the spinal cord by recording the electrical potentials developed in both the dorsal and ventral roots immediately adjacent to the spinal cord, a procedure first attempted by Gotch & Horsley and also employed by Umrath (54), Eccles & Pritchard (19), and Bonnet & Bremer (8). These roots must selectively lead out potentials developed in specific parts of the grey matter, for the slow potential waves are up to five times larger than those recorded with electrodes on the surface of the cord and are also much simpler. This selective leading has been shown to be due to electrotonic propagation along the nerve fibers forming the roots, the slow potential waves suffering a rapid exponential decrement along the roots in contrast with the constant size of the spike potentials produced by propagated impulses. Unfortunately there is much conflict of opinion in the interpretation of these electrotonic potentials, and the following detailed discussion is an attempt to clarify the position.

When in the cat an antidromic volley is backfired through an isolated ventral root into the motoneurones, electrodes on the root show that the spike potential of the antidromic volley continues on to a negative electrotonic potential, which after 10 to 20 msec. runs on to a positive electrotonic potential eventually disappearing at 70 to 200 msec. If the stimulus setting up the antidromic volley is weakened from a just maximal strength, the slow negative and positive electrotonic potentials diminish with the size of the antidromic spike, while strengthening the stimulus beyond maximal does not increase these potentials. Hence Eccles & Pritchard concluded that the slow potentials are produced in the motoneurones by the antidromic impulses, each impulse acting independently on its own motoneurone.

Barron & Matthews (6) have also recorded these electrotonic potentials, but on the contrary suggest that the antidromic

stimulus spreads by electrotonus along the ventral root into the spinal cord, producing the potentials by stimulating structures external to the motoneurones. This suggestion is based on their finding that no electrotonic potentials are recorded if the leads are applied to the ventral root in continuity, the antidromic volley then being set up by a stimulus applied much more distally, *e.g.*, to the sciatic nerve (dorsal roots cut). However, with such a disposition of the leads on the intact ventral root, the distal lead would act as a lead from the ventral root at its origin from the cord by virtue of the electrical connection made through the more distal part of the ventral root and the body of the cat back to the spinal cord. Thus with a suitable placing of the leads on the intact ventral root the slow potential variations at the proximal and distal leads would be practically identical, and so no potential change would be recorded. With similar leads from the intact ventral root Barron & Matthews also failed to detect the slow potential waves which an antidromic volley (antidromic stimulus applied to the sciatic) sets up in the motoneurones of the frog, though they record these potentials with two electrodes on the cord or even on an isolated dorsal root (see also 54). (There can here be no question of the production of these potentials by the excitation of structures external to the motoneurone by the electrotonic spread of an antidromic stimulus applied as remotely as the sciatic nerve). This failure to record with the ventral root leads the potentials arising in the frog's motoneurones obviously arises, as with the cat experiment, on account of a particular disposition of the electrodes on the intact ventral root. Thus in a similar experiment, but with presumably different positions of the leads on the intact ventral root, Bonnet & Bremer (8) found that an antidromic volley produces a slow negative potential as large as 1 mv. The improbability of Barron & Matthews' view that excitation is produced by the electrotonic spread of the antidromic stimulus to the cat's cord is further instanced by recalling that the slow potentials run parallel with the size of the antidromic volley, and that increase of the antidromic stimulus beyond the maximal for the ventral root fibers gives no further increase in the slow potentials.

Thus it must be concluded that the electrotonic potentials of the ventral root are produced by antidromic impulses, and presumably arise primarily in the motoneurones, there being a dimi-

nution in the polarization of their surface membranes during the negative wave, and an increased polarization during the positive wave. Such an action of the antidromic volley on motoneurones parallels closely its action on the ganglion cells of the superior cervical ganglion (14), but the time course is about five times faster and the negative wave is larger.

In the cat or the frog a volley of impulses entering the cord by the dorsal root also gives rise to a negative potential, its duration in the cat being 100 to 200 msec. In contrast with the ventral root, this negative potential is propagated electrotonically along other dorsal roots, both ipse- and contra-lateral, as well as the root of entry. Barron & Matthews suggest that these potentials occurring in the dorsal root fibers within the cord arise primarily in the central terminals of these fibers, being perhaps highly developed negative after-potentials, and they show ingeniously how such potentials could spread by ionic diffusion to adjacent passive fiber terminals, thus accounting for the experimentally observed occlusion of the electrotonically conducted potentials which are produced by volleys in different dorsal roots. However, such an explanation in terms of ionic diffusion can hardly be entertained for Barron & Matthews' own experiments, for they have shown that the electrotonic potential in the dorsal root by which the volley enters the cord is little if any larger than that in adjacent passive roots. No such difficulty arises, if, as suggested by Bonnet & Bremer (8), the dorsal root volley produces the negative potential (presumably a partial depolarization) in the neurones on which it impinges, and the resulting potential gradients affect secondarily all nerve fibers, both active and passive indiscriminately, which enter into close proximity with those neurones, particularly into synaptic relationship. The observed degree of spread between adjacent roots would then be no more than would be expected from the known degree of overlap in the central distributions of adjacent roots. Moreover the production of large slow potential changes by the cell bodies of neurones has already been established for ganglion cells bombarded by preganglionic impulses, and for motoneurones acted on by antidromic impulses; whereas the primary production in the nerve fibers of such large slow potential waves (up to 10 mv.) postulates a property for which there is no good evidence. All the other experimental evidence also is in conformance with the conclusion that the potential

change is produced in the neurones and electrotonically propagated along all fibers in close proximity to these neurones.

A dorsal root volley also sets up a slow negative potential conducted electrotonically along the ventral roots (5, 6, 19, 8). In conformity with their hypothesis, Barron & Matthews assume that this negative potential is primarily produced by nerve terminals ending in synaptic contact with the motoneurones, which are only secondarily involved. However, we have seen that motoneurones respond to an antidromic volley by slow negative and positive potential waves; and, when a dorsal root volley sets up a large motoneurone discharge, a positive potential wave of similar shape and duration also may be observed to be superimposed on the prolonged negative background of the ventral root electrotonic potentials (19), an observation supporting the view that an antidromic impulse and the discharge of an impulse by a nerve cell have identical actions on that cell. There can be no doubt that these slow potentials arise primarily in the motoneurones; hence these is every probability that impulses entering into synaptic relationship with motoneurones produce a slow negative potential arising primarily in those motoneurones and being secondarily propagated electrotonically along the motor nerve fibers of the ventral root. The complex shape of this negative electrotonic wave (often with early and delayed crests) indicates that a single dorsal root volley gives rise to a prolonged irregular bombardment of the motoneurones.

The intermediary potentials led from the dorsum of the cord give evidence of a complexity of behavior of internuncial neurones which would be adequate to account for this prolonged bombardment. The part played by internuncial neurones in conditioning responses of the oculomotor neurones has recently been subjected to detailed experimental analysis and correlation with their anatomical connections by Lorente de Nó (40). His conclusions would also for the most part, obtain for the internuncial neurones of the spinal cord, though his neglect of the excitability changes which accompany the slow potential waves would disallow their complete acceptance. However, they serve to illustrate in a more precise manner the previous description (15, pp. 384-7, 388-91) of the functional interconnection of internuncial neurones during the complex intermediary action potentials.

## EXCITATORY REACTIONS OF NERVE CELLS

There is now conclusive evidence that nerve impulses impinging on a nerve cell at synapses (*i.e.*, synaptic stimulation) may excite that cell to discharge an impulse either (1) by a brief excitatory action, or (2) by a later prolonged excitatory action (the "central excitatory state") associated with a relative negativity (*i.e.*, partial depolarization), of the cell body relative to its axon. When the spinal cord is chronically isolated so that it is freed from all incoming impulses, Tower (53) has shown that there is no spontaneous discharge of impulses, *i.e.*, in the absence of synaptic stimulation, and hence of the above excitatory actions, nerve cells are unable to discharge impulses.

(1) *The detonator action.*—The existence of the brief excitatory action (called the "detonator action") was originally described and investigated by Lorente de Nó (36) in oculomotor neurones, and by Eccles (15, 16) in ganglion cells, and the assumption that it was present in the motoneurones of the spinal cord was only established when large dorsal root volleys were found to set up a discharge from motoneurones with the very short synaptic delay of 0.55 to 0.8 msec. (19, 52), values identical with those observed by Lorente de Nó (38) for oculomotor neurones. These brief synaptic delays were also observed in the spinal cord by Barron & Matthews (6), who, however, suggest that such responses are experimental anomalies resulting from the direct spread of the dorsal root stimulus to "central structures" (unspecified) in the cord, for such responses with brief latency were not observed when a large peripheral nerve was stimulated or even when the dorsal root was stimulated more distally (2 cm.) from the cord. However, Lorente de Nó (38) has shown that with an oculomotor neurone the detonator action is very brief, summation between the detonator actions of different impulses rapidly undergoing decrement and finally disappearing when they are dispersed over a range of only 0.3 to 0.4 msec.; thus the increased temporal dispersion of impulses resulting from the more distal application of the stimulus might well explain the disappearance of responses of brief latency as observed by Barron & Matthews. Moreover, Lorente de Nó's (37) observations on direct stimulation of the oculomotor nuclei show that motoneurones themselves respond to direct stimulation with no appreciable delay; hence, even if Barron & Mat-



thews were correct in ascribing the short latent periods to spread of the stimulus to "central structures" in the cord, these "central structures" could be no further downstream than the nerve fibers entering into synaptic relationship with the motoneurons, it being thus still necessary to recognize the existence of synaptic delays of less than 1 msec., *i.e.*, the detonator action at synapses.

On either the chemical or the electrical hypothesis of synaptic transmission, the excitation (*i.e.*, the detonator response) produced by synaptic stimulation must be confined initially to an area on the surface of the motoneurone but little larger than the synapse. In view of the spaces separating synapses (28, 39) the experimentally observed summation of the subliminal excitatory actions produced at different synapses indicates that there is a decremental propagation of each detonator response away from the synapse of its origin. Now for peripheral nerve Rushton (46) and Katz (34) have recently shown that on the electrical theory of the propagation of the nerve impulse there must be a certain minimal length of excited nerve before propagation can occur as an all-or-nothing process. With a shorter length of excited nerve, propagation of the excitation is decremental and rapid extinction occurs (27) giving a response which Lucas called the local excitatory state. It may be taken as established that nerve impulses are propagated over the surface of a nerve cell exactly as along peripheral nerve fibers (14; 15, p. 353), hence Rushton's concept of a minimal critical length of excited nerve must be extended to the surface of the nerve cell, a minimal critical area of excitation being necessary for initiating the propagation of an all-or-nothing impulse over the surface of the cell body and along the axon of the nerve cell, *i.e.*, for the discharge of an impulse by the cell. The excited area at any one synapse would be far less than this minimal critical area, hence it would be extinguished after a small decremental spread, there being presumably a still further surrounding area of electrotonic spread as in peripheral nerve (25, 26).

On this basis an explanation is provided for all the observed features of the detonator response. Thus detonator summation results from the fusion both of the excited and of the electrotonic areas which spread decrementally from adjacent synapses, a sufficient area of excitation thus being obtained for initiating the all-or-nothing propagation of the impulse (see also 29). Such sum-

mation would of course only occur if adjacent synapses are excited within the very short intervals during which these decrementally spreading excitations survive. It has already been suggested (15, pp. 379-83; 16) that synaptic delay may be explicable by the time necessary for the spread and summation of the detonator responses of neighbouring synapses. Moreover the observed density of synaptic knobs (the synaptic scale of Lorente de Nó) over the surface of motoneurones (28, 39) would accord well with such an explanation, and Lorente de Nó has obtained evidence indicating that the excitation of strategic zones of synapses is essential for evoking the discharge of an impulse, his figures 3, 4 and 5 (39) illustrating in a particularly striking manner the extent of the synaptic contacts made by any one nerve fiber on a single nerve cell and the close relationship which these synapses bear to those of other fibers. If the detonator response is thus the small decrementally spreading impulse surrounded by a zone of electrotonus, there should be an associated electrical response. None has yet been detected, but it probably would not be distinguishable from the spike potential produced by the all-or-nothing propagated impulses in other neurones. In this connection it is of interest to refer to the recent observation (18) that a nerve impulse acting on a curarized muscle fiber may be set up a decrementally spreading impulse which is associated with a monophasic spike potential, closely resembling that obtained by Hodgkin (27) with subliminal stimulation of a single axon of crab nerve.

(2) *The central excitatory state.*—Earlier experiments showed that reflex facilitation depended on some enduring central excitatory process (10). Later evidence has shown that negative potential changes in isolated ganglion preparations were associated with the discharge of impulses (1), and that preganglionic impulses produced a slow negative potential of sympathetic ganglion cells and a corresponding lowering of their threshold to later synaptic stimulation (12, 13). Finally we have seen in the previous section of this review that synaptic stimulation produces a slow negative potential (partial depolarization) of the motoneurones of the spinal cord. Barron & Matthews (5, 6) in particular have established the causal relationship of this potential to the discharge of impulses from the motoneurones, their experiments being so convincing that they have been led to underestimate the importance of the detonator action described above. They have shown that

this depolarization is produced by all forms of reflex stimulation, and is progressively built up to a steady level during tetanic synaptic stimulation, the number of motoneurones discharging and the individual frequency of their rhythmic discharge depending on the degree of depolarization. Furthermore they have shown that the partial depolarization produced by the application of a weak steady direct current has caused motoneurones either to discharge impulses when previously quiescent or to increase their previous rate of discharge, while application of a current in the reverse direction has diminished this rate of discharge. It would thus seem that a certain degree of depolarization is in itself sufficient to provoke the discharge of an impulse from a motoneurone, and this discharge may be repetitive if the depolarization is sufficiently long continued.

In contradistinction to the detonator response the central excitatory state is associated with a relatively large negative potential, the size of the electrotonically propagated potentials in the ventral roots indicating as much as 10 mv. depolarization of the cell bodies of the motoneurones. Presumably such large and prolonged potential changes involve more or less uniformly the whole surface of the cell body, their distribution tending to be independent of the site of the originally excited synapses. Barron & Matthews' (6) experiments suggest that impulses are spontaneously generated by the motoneurones when this depolarization surpasses a critical value, but it must be remembered that similar results would be obtained if the depolarization merely lowered the threshold of the motoneurones to the detonator action of impulses, which action itself was actually the agent firing off the discharge. It is possible that the central excitatory state is analogous to the supernormal state associated with the negative after-potential of peripheral nerve, particularly if the subliminal detonator responses are, as suggested above, abortive impulses. However, with ganglion cells it has been shown that an antidromic volley produces much less central excitatory state than a preganglionic volley (14); hence synaptic stimulation of nerve cells would seem to have some action in producing central excitatory state additional to that resulting from abortive or full-sized impulses in those cells.

These two excitatory actions which are exerted by nerve impulses acting at the synapses of motoneurones (*i.e.*, the detonator

response and the central excitatory state) are well illustrated by the response to a dorsal root volley (19). When the volley is small, it produces after a latent period of about 1 msec. a slow negative potential of the motoneurones rising gradually for several milliseconds. If sufficiently large, a discharge of impulses is evoked with a synaptic delay of about 3 to 5 msec., the larger the response, the shorter (within limits) being the delay. There is no evidence of detonator action. However, a still larger volley evokes a discharge with a synaptic delay of no more than 0.55 to 0.8 msec. (see also 52). This discharge must be due to the detonator action, for it occurs during the latent period of the slow negative potential.

Eccles & Pritchard (19) have shown that an antidromic volley sets up a wave of positivity (increased polarization) of the motoneurones, which is associated with a decrease in their excitability, and that, as would be expected, such a positive wave also is produced by the discharge of impulses from motoneurones. This positive wave persisting for 70 to 200 msec. provides an explanation of the rhythmic responses of continuously excited motoneurones. After the discharge of an impulse, the motoneurone's excitability is depressed, and a second discharge is set up as soon as the excitability has recovered sufficiently (cf. 15, p. 392-4). The subnormal state produced by the discharge of an impulse would also explain Bernstein's experiments (7) without assuming that the central excitatory state leads directly on to a subnormal state. Further in view of the interaction between the supernormal and subnormal states of nerve cells (13, 14, 15), there would now seem to be no necessity to postulate, as Kleyntjens (35) has done, that an antidromic impulse or the discharge of an impulse directly destroys the central excitatory state.

The complications introduced by internuncial neurones prevent any precise investigations into the interactions of the slow negative and positive waves and the associated changes in excitability of motoneurones such as has been possible with ganglion cells (12, 13, 14) but a similar behaviour is indicated by preliminary investigations with two antidromic volleys (19). With frog's motoneurones the interval between two antidromic volleys must exceed 7 msec. before the second volley adds anything to the slow negative wave. Bonnet & Bremer (8) regard this interval as a measure of the absolutely refractory period of the motoneurones, but comparison with the cat experiments indicates that it is more

likely to be an extreme degree of occlusion between the negative waves set up by the successive antidromic volleys, the absolutely refractory period of motoneurones being probably no longer than that of their axons (37).

We have seen that the dorsal root potentials are led off selectively, presumably from the internuncial neurones. A similar occlusion to the above is observed between the negative waves set up by successive volleys. It would thus seem that the internuncial neurones behave similarly to motoneurones, though there is but little sign of a later positive wave in the potentials propagated electrotonically along the dorsal roots (6, 8). Perhaps these positive potentials are submerged by the negative potentials continuously being produced by the bombardment of the internuncial neurones by impulses from delay paths in the cord (40). Histologically, too, there would appear to be little to distinguish the synapses of motoneurones from those of internuncial neurones (39); hence it appears probable that all nerve cells exhibit in general a similar behaviour.

#### THE DISCHARGE OF IMPULSES FROM MOTONEURONES

Opinions differ greatly with regard to the relative significance of the detonator response and of the central excitatory state in evoking the reflex discharge of impulses. Thus Lorente de Nó (40) states that he has no certain evidence for the existence of central excitatory state in his experiments on oculomotor neurones and explains all reflex activity, including rhythmic responses and inhibition, in terms of the activity of chains of internuncial neurones, the impulses at every synapse exerting only the brief detonator action. On the other hand Barron & Matthews (6) and Bonnet & Bremer (8) regard the depolarization of the central excitatory state as being responsible for all reflex discharges. The above discussion has indicated that both factors play a part (see also 15), the motoneurones of the spinal cord thus resembling ganglion cells (16). It is doubtful if the bombardment of motoneurones ever naturally reaches the intensity and synchronism that is necessary for the detonator responses to evoke a discharge from resting motoneurones, but, when the threshold is lowered by the central excitatory state generated by previous synaptic stimulation, then the detonator responses may well reach threshold intensity, as is

shown for example by the very short synaptic delay of a facilitated reflex response (20, 35).

The slow negative potential produced in motoneurons by an antidromic ventral-root volley is associated with an increase in their excitability (35) which often leads to a brief repetitive discharge of impulses from some motoneurons at about three hundred per second (19). This discharge is increased during the larger negative potential which arises when two antidromic volleys are fired at a short interval, and diminished or abolished when the negative potential reaches a lower absolute value on account of its superposition on the slow positive wave produced by an earlier antidromic volley; hence it may be concluded that the partial depolarization of the motoneurons, as evidenced by the negative potential, is responsible for evoking the repetitive discharge from some of them.

#### THE DISCHARGE OF IMPULSES ALONG DORSAL ROOTS

There is much difference of opinion regarding the course of the nerve fibers of the dorsal roots [cf. the recent summary of the situation by Fulton (21, p. 32-4)]. A critical evaluation of the conflicting investigations is rendered difficult on account of the differing methods and animals which have been employed; nevertheless, the following three types of fiber may be recognized:

(a) The normal sensory fiber with its cell station in the dorsal root ganglion, from which the fiber passes in the dorsal root to terminate in the central nervous system by branches entering into synaptic relationship with numerous nerve cells. These fibers form a very high proportion of the total.

(b) Fibers with their cell station in the spinal cord, and which anatomically may be regarded as efferent fibers in the dorsal root. If large fibers of this type exist, they are so uncommon that they have no functional significance (24, 51, 56). Small fibers (less than  $3\mu$ ) probably exist, but can form no more than a very small fraction of the total number of fibers (56).

(c) Fibers which are collaterals arising from the intraspinal pathway of type 'a' fibers, and which usually pass out in dorsal rootlets adjacent to the parent fibers. The degeneration experiments of Barron & Matthews (3) suggested that about 30 per cent of the fibers in the dorsal root belonged to this type, but counts of dorsal root fibers and ganglion cells show that this value must

be much too high (2, 11), the error possibly arising on account of the uncertainty in distinguishing normal from degenerated fibers in transverse sections. At most such recurrent collaterals can form but a very small fraction of the dorsal root fibers, a conclusion confirmed by the careful studies of teased preparations by Young & Zuckerman (56).

Barron & Matthews (3) produced convincing evidence that impulses entering the cord by a dorsal root fiber may leave the cord by another dorsal root fiber. The central pathway shows no more delay than would be consumed by impulse conduction time, and is capable of transmitting impulses at rates up to 600 to 700 per second. Their conclusion that there is direct continuity between the two dorsal root fibers, one being a collateral of the other, *i.e.*, a fiber of type 'c' above, would appear to be inescapable. Their physiological studies do not suggest that these fibers form more than the very small percentage that has been concluded to be present from the anatomical studies. Moreover the failure of Tönnies (52) to detect such discharges indicates their rarity, since more than a very few impulses would certainly be detectable even in the whole roots that Tönnies recorded from. Alternatively it might be suggested that transmission of the impulse could occur from one dorsal root fiber to another by direct electrical spread, such as has been observed by Jasper & Monnier (33) with the non-medullated nerve fibers of invertebrates, but with such transmission there was a delay much longer than could have occurred in the above observations of Barron & Matthews.

In the dorsal root Barron & Matthews (3) have also shown that a dorsal root volley sets up the discharge of impulses which were apparently relayed in the spinal cord, and recently this type of discharge has been fully investigated by Tönnies (52), whose results may be tabulated as follows: (1) A single dorsal root volley evokes the discharge both in the stimulated root and in the neighbouring roots, the size of the discharge diminishing progressively with more distal roots and also being much less on the contralateral side. (2) Impulses in the large dorsal root fibers are most effective in evoking the discharge, but the smaller fibers also probably play a part. (3) The discharge of impulses has been detected in all fibers of the root except the C fibers, and impulses in large fibers are effective in evoking the discharge of impulses in fibers of all sizes—even the small  $\delta$  fibers. (4) The short-



est time between the entry of impulses into the cord and the discharge of impulses therefrom is about 4 msec., and is independent of the size of the afferent volley. With contralateral responses the minimum central delay is at least 1 msec. longer. (5) The discharge continues for about 20 msec., but to a large extent this is due to repetitive discharges, the intervals between successive discharges being 2.5 to 3.5 msec., and there is no evidence that the initial discharges in some fibers can be delayed to this degree. (6) The discharge occurs in a very high proportion of the dorsal root fibers, a conservative estimate being 35 per cent. (7) If two afferent volleys are almost synchronous, there may be summation of the discharge they evoke. On the other hand there is inhibition of the response to a testing volley even when this volley is as early as 4 msec. after the conditioning volley. This inhibition reaches its maximum with a volley-interval of 10 to 50 msec., and recovery is almost complete in 300 msec., though more than one second is needed for complete recovery.

We have seen that in the dorsal roots there are few, if any, large fibers with their cell station in the spinal cord (the above type 'b'); hence there is no escape from Tönnies' conclusion that the efferent discharge occurs in fibers whose cell stations are in the dorsal root ganglia. Tönnies further concludes that the discharge is a reflex response comparable with that occurring along the motor fibers of the ventral root, and on the basis of the long central delay suggests that as many as five or six synapses are traversed even with the quickest ipsilateral responses, and this pathway would not be shortened for facilitated responses. This in itself seems improbably long for the shortest central pathway from dorsal root fiber to dorsal root fiber, and the observed rate of efferent discharge, 400 to 300 a second, is also improbably high for a true reflex.

However this discharge of impulses along the dorsal roots must be considered in relation to the other response of the dorsal root fibers, namely the negative electrotonic potential. However produced, this electrotonic potential is so large in the dorsal root fibers at the surface of the spinal cord that Barron & Matthews (6) estimate that the fiber terminations in the grey matter of the cord must develop a negative potential of as much as 10 mv., *i.e.*, there is a depolarization by as much as one-third of the resting demarcation potential; and the shape of the electrotonic potential

shows that the greater part of this central depolarization must occur in a few milliseconds. Now Hodgkin (26) has shown that a brief depolarization of 2 to 3 mv. is sufficient to set up an impulse in a peripheral nerve fiber; hence, on the assumption that their central terminals behave as ordinary nerve fibers, their estimated depolarization (increasing to 10 mv. in a few milliseconds) would be expected to evoke the discharge of impulses along the dorsal root fibers. Furthermore, in all respects this depolarization shows just those features which would account for all Tönnies' observations on the dorsal root discharge. Thus a dorsal root volley produces a larger negative electrotonic potential in its own root or in adjacent roots than in more distal roots, and the potential is still smaller in contralateral roots (cf. section 1 of tabulated results). The approximate latency measurements of the electrotonic potential (6) as well as the longer and slower negative potential on the contralateral side agree with section 4 of the above tabulated results, while section 5 of these results corresponds in rate and duration with the repetitive discharge that would be expected during the brief rising phase of the negative wave. Summation of electrotonic potentials has been observed for simultaneous dorsal root volleys, but the second of two successive volleys produces a much smaller potential than when alone, the volley interval affecting this depressant action much as it affects the inhibition of the discharge (cf. section 7 of the tabulated results). The general involvement of all the fibers of the dorsal root and the large proportion of these fibers carrying efferent discharges would also be expected on the above explanation (cf. sections, 2, 3 and 6 above).

It may therefore be concluded that the efferent dorsal root discharges observed by Tönnies most probably result from the partial depolarization of these fibers in the grey matter of the spinal cord. It was concluded in the first section of this review that this depolarization is probably due to secondary involvement of these dorsal root fibers by the slow negative potential produced primarily by the nerve cells with which they enter into synaptic relationship. Thus, on this explanation, the efferent discharges in the dorsal roots occur along the ordinary sensory fibers, being set up by a process which differs fundamentally from that described above for the true synaptic transmission which occurs in the reverse direction.

There can be no question but that these hitherto unsuspected

discharges along dorsal root fibers must considerably modify the interpretation of all previous reflex experiments on the interaction of two or more afferent nerve volleys. Presumably the central reflex effects of the conditioning volley would be considerably increased and prolonged by the impulses secondarily arising in the collateral branches of some dorsal root fibers and extending to other collateral branches of these fibers. On the other hand an afferent testing volley even at twenty or more milliseconds after the conditioning volley would be diminished by collision with efferent impulses in the dorsal root fibers; while its aggregate central bombardment would presumably also be below its control value on account of the production of a smaller secondary discharge at intervals even as long as one second after the conditioning volley. Hence arises one factor contributing to inhibition. Again if, as argued above, the efferent discharge in dorsal root fibers is produced by the negative potential arising in internuncial neurones subjected to a synchronous synaptic bombardment, then it is probable that this retrograde excitation also involves those internuncial fibers making synaptic contacts with these neurones; and furthermore other internuncial neurones and even the motoneurones may develop sufficient negative potential to produce this retrograde excitation in fibers making synaptic contacts with them. Thus it is evident that retrograde excitation might contribute to a widespread distribution throughout the spinal cord of the excitatory effect produced by a dorsal root volley.

However, in the normal functioning of the central nervous system, this retrograde excitation may be so rare that it is without significance. The practically synchronous synaptic bombardment of a nerve cell by impulses in several nerve fibers may be necessary to produce a rise of negative potential sufficiently steep to give rise to retrograde excitation. Certainly under normal physiological conditions it would be rare to find receptor organs firing into the central nervous system a volley that exhibited a degree of synchronism approaching that of a dorsal root volley set up by direct nerve stimulation.

Even the slow negative potential which an antidromic volley produces in motoneurones does not seem to set up impulses in the fibers (largely internuncial) ending in synaptic relationship with those motoneurones, for such impulses, spreading through the field of distribution of the internuncial neurones, would be ex-

pected to evoke the reflex discharge of impulses from other motoneurons and this has never been observed. In the frog (but not in the cat) the slow negative potential produced in motoneurons by antidromic impulses is even propagated electrotonically along the dorsal root fibers (54, 6), possibly because many of these fibers end synaptically in relationship with motoneurons.

#### INHIBITION

Widely diverse views have recently been expressed on the nature of reflex inhibition, and there can now be no doubt that many different processes may play a part, recent experiments demonstrating at least four.

(1) Barron & Matthews (3, 6) have shown that in the spinal cord there may be an intermittent blocking of impulse transmission in nerve fibers, probably by the slow potential changes which are electrotonically transmitted from their collaterals entering the grey matter (cf. the above dorsal root potentials). This intermittency is related to the action of afferent impulses in the grey matter, stimulation of an afferent nerve being found either to cause or to release the block. On this view inhibition would not be a synaptic phenomenon, but would be due to a failure of impulses to reach the synaptic endings either on the internuncial neurons or the motoneurons. Though such a process of axonal blocking must undoubtedly modify the reflex responses of the spinal cord to the afferent nerve impulses, it is difficult to believe that co-ordinated activity of the cord could thus be produced by a process which essentially depends on what presumably are the chance anatomical relations of the collateral branches to the parent fiber.

(2) The subnormal excitability of nerve cells which is associated with the slow positive potential (increased polarization) has been regarded as responsible for inhibition (30, 31, 32, 13, 21). In particular the depression of internuncial neurons has been shown to be related to inhibition by Hughes & Gasser (31) and Hughes, McCouch & Stewart (32), and to have a time course and magnitude closely resembling the positive intermediary potential. It has not been demonstrated that a slow positive potential of nerve cells, *i.e.*, increased polarization, arises except as a result of the discharge of an impulse by that cell (6, 8, 19), the positive potential thus resembling the positive after-potential of peripheral

nerve. However, it has been shown that, by postulating a certain arrangement of internuncial neurones in the reflex pathway, such a depression following the discharge of impulses by internuncial neurones could account for the experimental observation that motoneurones may be inhibited even when they had not discharged an impulse (31, 22).

(3) The demonstration of the very short period during which there is summation of the detonator action of impulses impinging on a neurone at different synapses has led Lorente de Nó (40) to suggest that inhibition of a reflex response could occur by fractionation of an afferent volley into two or more volleys impinging on a neurone at intervals too long for such summation to occur (cf. 22). Such a mechanism has been illustrated by ingenious diagrams of possible neuronal interconnections, but it is difficult to accept an explanation in which in these complicated networks the arrivals of impulses at synapses are synchronized to a small fraction of a millisecond. Further, when the asynchronous nature of the normal discharge of impulses from receptor organs into the central nervous system is considered, the difficulties confronting such an explanation of inhibition appear to be grave.

(4) A contributory factor to inhibition has already been considered in discussing Tönnies' experiments on the discharge of impulses along the dorsal roots.

Under normal physiological conditions the reflex response evoked by the asynchronous repetitive discharge of impulses from one set of receptor organs may be inhibited by a similarly asynchronous discharge from another set of receptor organs, *e.g.*, the stretch reflex evoked in an extensor muscle by the discharge of impulses from its stretched muscle spindles is inhibited by the nociceptive sensory discharge set up by pinching the foot. In such an inhibitory action there would seem to be no possibility of the accurate timing which is essential for the above third explanation of inhibition and the fourth would also have little or no significance. Further, the second explanation appears to be but little more satisfactory if the subnormal excitability is restricted to those neurones which have discharged impulses, and both it and the first explanation do not seem able to account for the observed completeness of the inhibition. Thus inhibition of extensor reflexes appears to be inexplicable by any of the above four possible inhibitory mechanisms. The absence of direct evidence for the

existence of specific inhibitory synapses must not be taken to exclude their presence (cf. 15, pp. 396-7).

#### THE SYNAPTIC TRANSMITTER

Several recent investigations have been prompted by the view that the hypothesis of acetylcholine transmission at ganglionic synapses might also be extended to the synapses in the central nervous system. For example, Schweitzer & Wright (47, 48, 49, 50) have studied the action of acetylcholine and of anti-cholinesterases on the knee jerk, and have attempted to explain some of the effects produced by anti-cholinesterases as due to the accumulation of acetylcholine at synapses, thus supporting the hypothesis that acetylcholine is liberated at synapses during reflex activity. However, it is in any case necessary to postulate a direct action of anti-cholinesterases on nerve cells, and this direct action might well cover all the observed effects, there being then no evidence of acetylcholine liberation at synapses. Again Nachmansohn (41, 42, 43, 44) has found that the concentration of cholinesterase varies widely in different regions of grey matter, but is always more than in the white matter; and he also states that in the embryo the concentration of cholinesterase runs parallel to the development of synapses. Such observations could only be used to support more direct evidence in favour of acetylcholine transmission at synapses, and this evidence is lacking for the central nervous system (cf. 21).

There would seem to be general agreement on the essential similarity of the transmission processes at the synapses in the sympathetic ganglia and in the spinal cord. With sympathetic ganglia Rosenblueth & Simeone (45) claimed to have shown that anti-cholinesterases slow the rate of decay of the synaptic transmitter, but their experiments were vitiated by an unsuspected complication (17), and the earlier observations of Eccles (16) still stand as a demonstration of the absence of any detectable action of anti-cholinesterases on the detonator action at ganglionic synapses, and hence of the unlikelihood of acetylcholine being the synaptic transmitter. No such experiment has yet been made with synaptic transmission in the spinal cord, but Bremer & Kleyntjens (9) have found that eserine apparently does not prolong the duration of the central excitatory state of the spinal cord, an action which would be expected if acetylcholine were the synaptic transmitter (cf. 15, pp. 366-7).

The narrow time limits 0.5 to 0.9 msec., which Lorente de Nó (38) has now placed on the synaptic delay for oculomotor neurones show that the synaptic transmitter very rapidly disappears, a condition which must obtain if this transmission is actually effected by the action currents of impulses impinging on the nerve cell at synapses. On the other hand, by making several assumptions, possibly unjustifiable, Nachmansohn (43) has calculated that such a rapid removal of the hypothetically liberated acetylcholine could be brought about by the cholinesterase in the grey matter of the central nervous system. In view of the paucity of experimental data further discussion of these rival hypotheses of synaptic transmission—the chemical and the electrical—is unwarranted.



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KANEMATSU MEMORIAL INSTITUTE OF PATHOLOGY  
SYDNEY HOSPITAL,  
SYDNEY, AUSTRALIA

## BIOELECTRIC STUDIES OF THE EXCITATION AND RESPONSE OF NERVE\*

BY DETLEV W. BRONK AND FRANK BRINK, JR.

*Eldridge Reeves Johnson Foundation and the Institute of Neurology,  
University of Pennsylvania, Philadelphia, Pennsylvania*

Studies of bioelectric phenomena in nerve serve two general objectives. The action-potential which accompanies the nerve impulse is useful as a mere indicator of nervous activity and is therefore a valuable means for investigating the functional organization and the reflex action of the nervous system. Even more significant is the index which the potential changes provide of the nature and time course of the fundamental cellular processes which constitute the activity in each unit.

In reviewing the literature on this subject which has appeared between January 1937 and June 1938 we have selected only such material as could be related to a connected account of our present knowledge of the mechanisms of excitation and conduction in nerve. Because reflexes and brain potentials are discussed in separate chapters the use of bioelectric activity for analyzing the organized patterns of the nervous system is not considered here. We have however given special attention to recent work dealing with those properties of nerve which determine the functional relations of one neuron to another. A knowledge of these characteristics and of the time course of their variation is essential to an ultimate understanding of how the activity of nerve cells is integrated into the behavior of the cellular aggregates which comprise the central nervous system.

### THE EXCITATION PROCESS

The most satisfactory method for the experimental stimulation of nerve is by means of an electric current. It is thus possible accurately to grade the intensity of the stimulus and determine its time course in a manner not feasible for other physical or for chemical agents. The immediate effect of the current is a movement of ions, and this has an important influence on the molecular structure of the protoplasm and its surface properties. If the intensity of the stimulus be sufficient, the molecular and ionic

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relations of the nerve are so altered that a propagated impulse is developed. Before that is achieved, however, or as a result of subthreshold stimuli, important changes take place in the protoplasm.

It is characteristic of nerve, as of other cells, that there normally exists a potential difference across its surface boundary. This demarcation potential is generally attributed to the differential restrictions imposed by the surface layer of the cell on the movement of positive and negative ions. It has long been assumed that the mechanisms of excitation and conduction are related to the properties of the nerve which give rise to that potential difference. It is significant, however, that the excitability may vary independently of the demarcation potential, and Curtis & Cole (1) have recently failed to find a change in the transverse impedance of the giant axon of the squid when it becomes completely inexcitable.

Wilbrandt (2) has studied the changes in the demarcation potential of myelinated and unmyelinated nerve fibers produced by an homologous series of organic anions. The changes were definite but small, and he concludes that the metabolic production of such ions could account for relatively small, slow after-potentials but not for the rapid spike potential. Although this demarcation potential can be modified by external agents, it exists because of intrinsic cellular forces. It is, therefore, to be contrasted with a potential difference established by extrinsic factors.

Such a potential is the electrotonic potential produced by the passage of an electric current through a nerve. This is a polarization potential established across the surface. When the current is interrupted the potentials at the anode and at the cathode, each referred to some remote point, initially decrease as though a condenser were discharging. This fall of potential is called the direct wave by Strohl (3) and is the end of the process in the immediate region of the cathode [cf. however (4)]. At the anode, on the other hand, the fall of potential is more rapid (3), and the direct wave is followed by a prolonged potential of the opposite sign which makes the anodic region negative. This delayed wave at the anode depends upon the integrity of the membrane and has been attributed to the accumulation of negative ions at the inner surface. Fabre (5), however, has proposed an alternative explanation in terms of an electro-osmotic transport of water through the interstices of the protoplasmic gel. This dilution of peripheral

layers of the protoplasm is assumed to give rise to a diffusion potential associated with the equalization of ionic concentrations.

Since these potentials are due to ionic movements, it is to be expected that changes in the ionic environment will modify the electrotonic effects. For example, Chweitzer (6) has shown that alterations in the cation content of the environment affect primarily the lowering of threshold at the cathode, while changes in the anions modify the anelectrotonic increase of threshold. The rate constants of the excitatory processes are dependent upon these electrotonic potentials (7, 8) and Bouman (8) finds that this relation is influenced by the nature of the ionic environment.

As the strength of a stimulating current is increased there is a proportional increase of the electrotonic potential at both the cathode and the anode. When the current attains a certain definite value there is in the case of unmyelinated crab nerve an additional increment of potential at the cathode. This is described by Hodgkin (9) who shows that it is a local depolarization which is graded by the strength of the stimulus, after appearing at a definite threshold. The magnitude of this local potential decreases exponentially with the distance from the cathode (10), as does the electrotonic potential. Following a brief stimulus the potential rises to a maximum and then declines to zero in about 4 msec. (9). If the strength of the stimulating shocks be increased, the magnitude of the local response increases until finally at a critical value it develops into a propagated spike potential which is from three to five times as great as the initiating local response.

Since the local cathodal potential first appears at a definite strength of stimulus and has a graded magnitude, it resembles the inverse electrotonic potential wave at the anode which follows the break of a constant current (11). However, it is not known whether this anodal inverse potential has a causal relation to the propagated impulse which is developed under these conditions.

Katz (12, 13) has described experiments which suggest that a subthreshold stimulus sets up a local electric response in myelinated nerve. His conclusions are disputed, however, by E. A. Blair (14) who maintains that the smallest response of a myelinated fiber is that of one segment, and that it cannot be graded. There is accordingly no definite and generally accepted evidence that propagated impulses can be initiated in myelinated nerve by the development of a local electric response, as has been shown to be

the mechanism in the more unstable (15) crustacean nerves. The impulse is, however, initiated by the development of an electrotonic potential to a certain critical value. Furthermore, it has been shown (16, 17, 18, 19) that the depolarization which underlies the electrotonic potential must extend over a certain minimal length of nerve in order that the impulse may be propagated. When these conditions are satisfied there is some evidence of a localized decrease of impedance and of the demarcation potential (20) which are followed by the development of a propagated all-or-nothing impulse.

The propagation of the impulse has long been attributed to the stimulation of successive portions of the nerve by circulating currents associated with the activity. Although definite proof of this theory had been lacking, Hodgkin (21, 22, 23) has recently discovered important evidence in its favor. For he has shown that, when an impulse reaches a portion of the nerve blocked by cold or compression, the circulating currents extend beyond the block and set up in that region an electrotonic potential with an accompanying change of excitability. That such processes are responsible for the propagation of the impulse is given further support by the observation of Cole & Curtis (20) that the initial rise of the spike is a passive alteration of the potential difference which arises from current flow preceding the all-or-nothing changes of impedance and E.M.F. Thus, the mechanism of conduction is one aspect of the general problem of excitation.

The electrical excitability of a nerve fiber is an index of important characteristics of the nerve at any instant, including its capacity to receive and to transmit impulses. This excitability is measured in terms of the threshold strength of current necessary to initiate a conducted impulse. The irritability thus determined may fluctuate without relation to any known agent (15, 24, 25), and it is modified by various factors such as changes in the chemical composition of the axon or its environment [Lehmann (26)], previous activity, and the passage of a threshold or subthreshold current. This last is of especial significance because it concerns the normal mechanisms of excitation and conduction, and because it reveals certain important facts regarding the nature and time course of the excitatory process.

We have pointed out in previous paragraphs that the local excitatory process developed by a subthreshold current decays to

zero when the exciting current is interrupted. The rate of this decay is governed by a constant  $K$ . This is referred to as the excitation constant, and it has been shown (27) to be directly proportional to the chronaxie. If the excitatory process be maintained by a continued flow of current, the threshold of the nerve is increased due to a process of "accommodation"; if the current increases slowly, the threshold rises gradually as a consequence of, and at a speed determined by, the increase of local excitatory process. This is the explanation given by Hill (27) and Solandt (28) of the well-known fact that the threshold for a slowly increasing current is higher than for a quickly increasing one. The excitation process and the accommodation process are defined by constants which measure the rate of return of the state of excitation and the threshold respectively to their resting levels, rather than the rate of their development. They are accordingly characteristics of the tissue only and are not influenced by the form of the applied current.

There is at present a disagreement as to whether the time constant of the accommodation process is always proportional to the excitation constant (29, 30, 31, 32, 33) or whether the two can vary independently (27, 34, 35). This difference of opinion is in part due to a disagreement concerning the normal form of the accommodation curve (31, 36), which is the relation of the threshold intensity of an exponentially increasing current to its rate of rise (28). The experimental determination of these constants is further complicated by the dependence of threshold determinations upon electrode size and separation (36, 37). The issue involved in this controversy is whether the changes in the nerve associated with electrical excitation are governed by one fundamental time constant or by two independent ones.

Following the end of a subthreshold current there are changes of excitability which have an important bearing on the mechanism of spatial and temporal summation in the nervous system. For instance, the nerve in the region of the cathode has an increased irritability for a time known as the period of latent addition, following which there is a period of lowered excitability. At the anode the effects are opposite [Erlanger & Blair (38)]. Recently Gasser (39) has found that there is a second period of increased excitability which follows the two earlier phases and lasts for as long as 100 msec. He suggests that the increased excitability during



the classical period of latent addition underlies the type of summation produced by two volleys arriving almost simultaneously in adjacent synapses. The newly-found, second period of increased excitability is responsible for summation such as that in which each afferent volley produces a small increment of excitation, and only gradually is the maximum number of neurons brought into activity. Such a summation of relatively slowly recurring impulses at a partially blocked region of nerve has been observed by Blair & Erlanger (40), and Gasser (39) has found it possible to excite peripheral axons in the mammal with subthreshold shocks repeated at intervals as long as 100 msec. These phenomena can be attributed to the delayed period of increased excitability.

Thus far we have been concerned with the variations of irritability associated with the development of the excitation process to a level at which a propagated impulse is initiated. Following the conduction of the spike potential there is also a cycle of irritability changes which in certain respects resembles the course of that following a subthreshold stimulus. Thus the periods of latent addition and subsequent depression taken together may be considered to represent the spike process in subthreshold form. The second period of increased excitability would then represent the process which above threshold gives rise to the increased excitability and the associated negative after-potential (39). The relation of excitability to after-potentials implied in the last statement brings us to the subject of our next section.

#### AFTER-POTENTIALS

The analysis of the sequence of negative and positive potentials which follows the spike potential and its correlation with other aspects of nervous activity constitutes one of the most important recent advances in neurophysiology. An excellent account of the work done in this field through 1936 is given by Gasser in his Johnson Foundation Lectures (41) of that year.

The potential changes associated with a single "nerve action" in the fastest mammalian fibers are the spike which lasts about 0.4 msec.; a negative after-potential continuing 15 msec.; followed by a positive potential which does not subside for 80 msec. or more (42). In slowly conducting fibers the durations of these several phases are greater.

The magnitude of the positive after-potential in a mammalian

A fiber is about 0.2 percent of the spike, and that part of the negative after-potential which can be distinguished as a separate component is probably not more than 5 to 6 percent of the crest of the spike (43). The smallness of these potentials, the brief duration of the earlier components and the long duration of the later phases necessitate a recorder of high sensitivity, rapidity and stability. These severe requirements explain why this sequence of events has not been adequately investigated until recently.

The after-potentials are important because they are an index of the intimate mechanisms of certain nervous processes. Thus Gasser (41, 44) has pointed out their relation to increased metabolism and suggests a parallelism between them and the time course of heat production. This suggestion has been confirmed by Parkinson (45) who finds that the initial phase of the positive after-potential corresponds to the rapid phase of heat production when both are measured under similar conditions. Circumstances which increase the metabolism of nerve increase the after-potentials but have little effect upon the spike, and in asphyxia the after-potentials are abolished when the spike is still present (26). We may therefore conclude that the processes represented by the after-potentials may be necessary for ultimate complete recovery of the nerve structure, but are not essential to the reestablishment of conditions which make possible the conduction of successive impulses. However, the capacity of a nerve to conduct trains of impulses at a high frequency is seriously impaired by conditions which reduce the after-potentials.

Probably the greatest significance of this sequence of potentials is its relation to the irritability cycle (41, 42). Following the refractory periods a supernormal excitability supervenes and lasts 15 msec. in the fastest mammalian fibers. Finally, there comes a period of subnormal excitability lasting 80 msec. The period of increased excitability is concurrent with the negative after-potential, the decreased excitability with the positive after-potential. The duration and magnitudes of the potentials can be altered by various means but they still parallel the course of the irritability cycle.

Among the factors which modify the after-potentials is the pH of the surrounding fluids (46). For instance, an increased pH shortens both the positive and negative phases, while the depth and area of the former is increased and the area of the negative

potential is decreased. Assuming that these changes might be due to deionization of calcium Lehmann (47) has altered the ratio of calcium to potassium of mammalian A fibers by removing calcium from the bathing fluid or by adding citrate. This decreases the early negative potential and increases the positive phase, while removal of potassium has an opposite effect. Because the results of high pH and low calcium are similar it is concluded that alterations in the potential cycle of nerve in alkaline media are mainly due to deionization of the calcium, in acid media to ionization of the calcium compounds.

Since the after-potentials are related to recovery processes Lehmann's (26) studies on the effects of asphyxia are of interest. During the early stages of oxygen lack the negative after-potential of the peroneal nerve of the cat becomes smaller, and the positive potential is increased. If asphyxia continues, the negative after-potential disappears, while the positive potential increases, although finally this too is smaller. In the saphenous nerve, on the other hand, the decrease of negative after-potential and the increase of the positive are very much less, a condition which can be produced in the peroneal by bathing it in Krebs' solution containing no potassium and excess of calcium ions. From these observations we may conclude that marked differences in those properties of nerve which are revealed by the after-potentials may be due to differences in their supply of available ions. It also follows that changes in the properties of a nerve induced by asphyxia may be due to altered ionic concentrations.

The after-potentials are much altered by trains of impulses such as those which normally traverse nervous tissue (43, 48). During a tetanus both the negative and positive after-potentials increase, but the positive grows relatively more than the negative. As the frequency and duration of the stimulus is increased the positive after-potential is further augmented in size but not duration, and is followed by a second positive potential which increases in duration with the duration of the tetanus. This phase of the potential cycle may last for more than a minute (43). It is of especial interest to note that the conditions or processes which cause large after-potentials to appear during an action may persist for a minute or more following a tetanus.

From what has been said it will be apparent that these changes in after-potentials induced by various factors such as chemical

agents and activity are an index of altered irritability. As such they are determinants of the capacity of the nervous system for action. This has been well expressed by Gasser thus (42): "If the spikes may be called the message carriers of the nervous system, the after-potentials in contrast may be called the indicators of the readiness with which messages will be accepted."

#### CORD POTENTIALS

Because the usual mode of excitation of nervous tissue is by nerve impulses at a synapse, it is important to know whether the axons and perikarya of the spinal cord and ganglia have irritability cycles like those of peripheral axons, and whether they are similarly related to a cycle of after-potentials. Much evidence indicates that this is so. For instance, Gasser (42) recites many parallels between the course of excitability following activity in the cord and in the periphery. Inhibition of a spinal reflex, he says "appears at various intensities; it accumulates by summation of the effects of a succession of afferent volleys; it lasts 0.1 sec. or longer, and its subsidence follows a typical curve of decay. In these respects its behavior follows exactly that of the subnormal period of peripheral nerve, for in the latter the intensity and duration also depend upon the number of impulses which produces it, beginning with about 0.1 sec. for a single response."

The potential changes associated with central inhibition and facilitation have been recorded through electrodes applied directly to the surface of the cord (49, 50, 51, 52). It is thus found that a single afferent volley sets up a spike potential in the continuation of fibers in the dorsal column. This spike is followed by a negative potential which lasts 10 to 20 msec. and a subsequent positive phase continuing 100 msec. Gasser and his colleagues attribute the slow negative potential to temporally dispersed spikes in the internuncials, and the positive phase to positive after-potentials. In accordance with this view they find the internuncial neurones less responsive during the positive phase. This has been confirmed by Hughes, McCouch & Stewart (52) who have also reported that the phase of delayed negativity is concurrent with a period of facilitation. One may conclude from such studies that the relationships between potential cycle and irritability cycle are the same for internuncial neurones and peripheral axons. Hughes & Gasser (50, 51) do not say how much of the observed potentials is due to

internuncial axons and how much to cell bodies, but Hughes, McCouch & Stewart (52) conclude that the positivity is largely an inhibitory state in the internuncial perikarya in the region of both dorsal horns, while the negative potential is an index of an excitatory state in or on other perikarya in these same locations.

Barron & Matthews (53) have studied spinal potentials by leading from the posterior or anterior roots instead of from the cord directly. It is thus possible to record an electrotonic potential which becomes smaller as it spreads along the posterior roots from the central terminations of the fibers. The sign of this potential is always the same and is such that the electrode nearer the cord is negative. This is interpreted to mean that the terminations of the fibers are depolarized by a change in the ionic environment which results from the passage of an impulse. If the return to the original ionic distribution were slow, there would be a prolonged depolarization of each fiber. This, rather than asynchronous, brief activity in many units, is their explanation of the long-lasting potentials, and the hypothesis is supported by the observation that a steady state of depolarization can be produced by temporal summation of afferent volleys. Such graded depolarization in the central nervous system may be of great importance in the control of nervous activity.

The relation of these posterior root potentials to after-potentials in peripheral axons and to cord potentials observed by other workers is not clear. The authors say the negative potentials are produced by the same mechanisms responsible for negative after-potentials in nerve but, if that be so, it is surprising not to find the positive potentials which might be expected to arise in such fine terminations. This difficulty is probably related to the issue which has been raised concerning the site of origin of the cord potentials. Barron & Matthews assume that the posterior root potentials arise in the central terminations of the posterior root fibers, but Bonnet & Bremer (54, 55), who have used the same method of recording, believe they have thus led from the internuncials across a synapse. Both of these teams think they are observing the same process as that which gives rise to the potential recorded from the dorsum of the cord. Because Hughes & Gasser (50, 51) attribute that to activity of the internuncial neurones, they are in agreement with Bonnet & Bremer and opposed to Barron & Matthews. These differences of opinion are important because their reconciliation

through further experiments will provide a better understanding of the source of the potentials led from complex arrangements of nerve cells, and of the significance of various types of leads.

Similar observations of the electrotonic potential in anterior roots give information concerning the depolarization of motor nerve cells (53, 54, 55, 56). These potentials are in many respects similar to those recorded in the posterior roots: the central electrode is always negative, the potentials decline slowly and they can be increased by temporal and spatial summation although the degree of summation may differ in the two roots (compare 53 and 54, 55). Processes which produce reflex inhibition decrease the negativity, and in general the frequency of motor impulses in a reflex discharge is related to the magnitude of the depolarization. These potentials are therefore an index of the level of central excitation and are important clues to excitatory mechanisms. Adrian (57) has found a similar relation between the degree of activity in the optic ganglion of *Dytiscus* and the magnitude of its sustained negative potential which he considers to be due to a steady depolarization.

#### GANGLION POTENTIALS

Slow potentials have also been recorded from sympathetic ganglia and have been correlated with the excitability of the postganglionic cells. Here, as in the studies on the cord, there is no general agreement as to where the potentials arise. Eccles (58) and Lloyd (59) say that the cycle of potentials is in large part due to the somata of the ganglion cells. But Bronk *et al.* (48) have considered these potentials in relation to the after-potentials in the postganglionic nerve, and they emphasize the necessity for recognizing that slow potentials are developed in the intra-ganglionic postsynaptic fibers, and are recorded from the ganglion. They do however agree that the after-potentials in the ganglion are larger relative to the spike than in the case of postganglionic nerve. Bishop (60) has objected even more strenuously to the assumption that ganglion potentials are largely due to cell bodies, and he has been answered in turn by Eccles (61). This difficulty of deciding how much of the slow potential cycle is due to axon potentials and how much to changes at the surface of perikarya is in part due to the similarity of the course of the potentials in synaptic regions and in axons.

According to Eccles (62) the negative phase of the ganglion potential is associated with increased excitability, and the positive phase with inhibition or decreased excitability. This correlation, which agrees with that observed in peripheral axons and in the cord has, however, been denied by Rosenblueth & Simeone (63) who claim that facilitation and inhibition in the ganglion may be quite independent of the negative and positive after-potentials. This question of fact should be settled by further measurements, for it leaves in doubt the significance of slow potentials in ganglia and in the central nervous system as well. Relative to this question Rosenblueth & Simeone point out that a correlation between after-potentials and electrically determined irritability in peripheral axons implies no similar correlation between cell potentials and synaptic excitability, if synaptic transmission is humoral rather than electrical. Such a relation between the threshold of ganglion cells to acetylcholine or potassium and the potential cycle has been determined by Bronk, Larrabee & Brink (64, 65). They find that the response of the cells to these agents is greatly reduced during the positive after-potential which is developed in the ganglion by either a preganglionic or antidromic volley. These questions bring us naturally to our next section.

#### THE MECHANISM OF SYNAPTIC TRANSMISSION

Sympathetic ganglia are favorable preparations for the study of this problem because, without internuncial neurones, the preganglionic fibers make direct connections with cells, the axons of which run out into the postganglionic trunk. Ganglia have the further advantage of a circulation which can be easily isolated in order to control the environment of the cells and synapses and to analyze the perfusate for substances liberated by transmission processes. Much of the work reported in this section has, therefore, been done on such mammalian preparations.

A single volley of impulses coming into a ganglion initiates only a single volley of postganglionic impulses, with a temporal dispersion which might be due to differences in conduction time and synaptic delay and to a repetitive discharge from the individual cells. By recording impulses from a very few units Bronk, Tower, Solandt & Larrabee (48) have shown that repetitive discharge does not occur, for each ganglion cell discharges only one impulse in response to a single volley of preganglionic impulses.



Similar conclusions concerning the activation of certain motoneurons in the central nervous system may be drawn from the experiments of Lorente de Nó (66).

The altered state of the nerve cell which initiates the impulse is believed to develop as a local process under the presynaptic termination and to spread decrementally (67, 68, 69). In order that this process, described by Eccles as the "detonator response," may set up a propagated impulse down the axon there must be summation of the subliminal local effects produced at a sufficient number of active synapses.

The nature of this process which sets off the impulse is not defined, and it has not been identified by an electrical sign. Its magnitude and time course are, therefore, measured in terms of the threshold of the cell to excitation by a nerve impulse. In this way Eccles' (67) work on sympathetic ganglion cells and Lorente de Nó's (68, 69) on the trochlear motoneurons lead them to believe that the process rises to a maximum and then falls, the degree and rate of rise being determined by the number of synapses delivering impulses. As the intensity of the process reaches a certain value a propagated impulse is discharged. The time elapsed between that instant and the arrival of the incident impulse at the synapse is the time of synaptic delay. This term can, however, have meaning only by arbitrary definition. For inasmuch as a nerve cell is probably excited only by the activity of more than one synapse, it is necessary to specify the delay with regard to the arrival of some one impulse. And, because the impulse itself is not an instantaneous process, the delay must be defined relative to some phase of the chosen presynaptic nerve action. For the trochlear motoneurons the delay is given as 0.5 to 0.9 msec. (68); for the motoneurons of the spinal cord, 0.7 to 1.0 msec.; and for the most rapid cells of the superior cervical ganglion, 2.5 to 5.0 msec. (67). One reason for the range of values is that, if the rate of rise of the response is increased by increasing the number of active synapses, the threshold for impulse propagation is attained sooner.

Following the development of an impulse the "detonator response" is obscured by the refractory period. If, on the other hand, the peak of the response is not adequate to set up an impulse, the response rapidly decays. Because of this brief duration it is probably impossible to produce summation by successive impulses in the same fiber (66, 67). A neurone will respond, therefore, only when

sufficient impulses arrive at different synapses within the brief period of effective summation.

Among the current conceptions of how the presynaptic impulse produces the response in the adjoining nerve cell is the hypothesis that the transmission is effected by the action currents of the incident impulses. Supporting evidence is mostly indirect. For instance, Jasper & Monnier (70) find that an impulse in one non-myelinated crustacean nerve can excite fibers in an adjoining nerve trunk under certain favorable conditions, and this may be by means of action currents. The 20 msec. delay of transmission in these experiments is, however, quite different from that observed at a true synapse and makes the relation of the phenomenon to the normal mechanism uncertain. A similar influence of action currents on adjoining nerve fibers in the same trunk is discussed by Auger (71) following an early suggestion of Adrian (72). It is also argued by some that the action current hypothesis is supported by Hodgkin's observations (21, 22) which favor the electrical transmission of impulses along an axon. It must be remembered, however, that the protoplasmic discontinuity at a synapse introduces physical conditions not found in Hodgkin's experiments. One of the most generally employed arguments in favor of the electrical theory of synaptic transmission rests upon the fact that the time course of the local process which develops in the postsynaptic cell is about the same as the course of the action potential in the presynaptic terminations (58, 68). It is further stated that the short synaptic delay and the brief duration of the response process probably rule out a chemical transmitter such as acetylcholine (62, 67).

Nevertheless the most generally accepted explanation of synaptic transmission assumes that the presynaptic impulse liberates a small amount of acetylcholine, and this in turn sets up the excitatory process in the cell body (73). There are two principal lines of evidence for this hypothesis. The first is the fact that acetylcholine is indeed formed in a ganglion during preganglionic stimulation and in a muscle during stimulation of the motor nerve. Whether the acetylcholine is liberated at the presynaptic terminations and only there is, however, a question about which there are two opinions. Lorente de N6 (74) reports the formation of acetylcholine in a ganglion when either the pre- or post-ganglionic nerves are stimulated and he therefore concludes that acetylcholine formation

is not restricted to the synaptic junction. This agrees with the report of certain earlier workers that acetylcholine is liberated in a peripheral axon by the passage of an impulse. On the other hand, Gaddum *et al.* (75) find this in peripheral nerve only under the stimulating electrodes with very strong stimuli, and MacIntosh (76) fails to confirm Lorente de Nó's observations with antidromic volleys. Such conflicting reports emphasize the need for more information concerning the mechanisms involved in the formation of acetylcholine and its relation to the electrical processes in nerve (77).

The theory of neurohumoral transmission is furthermore supported by the fact that acetylcholine does excite nerve cells and muscle fibers. Contraction of the nictitating membrane has often been produced by perfusing the superior cervical ganglion with acetylcholine (73). Recently, Bronk, Tower, Solandt & Larrabee (48) have recorded the persistent, rhythmic discharge of impulses from individual ganglion cells during continuous perfusion with acetylcholine, and Brown (78) has observed a similar rhythmic excitation of single muscle fibers. The sequence of impulses is regular and, in the case of the ganglion at least, the frequency is determined by the concentration of acetylcholine.

Because a persisting concentration of acetylcholine causes sustained activity, the neurohumoral hypothesis must explain how a nerve volley initiates but a single response at a synapse or at a motor end-plate. The usual assumption is that the acetylcholine formed at the junction is rapidly inactivated by cholinesterase. It is, therefore, interesting to find that the concentration of the enzyme is about three times as high in the superior cervical ganglion as in the non-synaptic vagus ganglion or in the cervical portion of the sympathetic nerve, and that this excess disappears after degeneration of the preganglionic fibers (79). There is apparently a functional relation between the location of the esterase at the presynaptic terminations and its rôle of inactivating the transmitter at that site. But it is not yet known how it is liberated, and Eccles (62) doubts whether its concentration is adequate to inactivate the transmitter with sufficient rapidity.

If repetitive responses to single nerve volleys are prevented by the esterase, inactivation of the esterase by eserine or prostigmin should permit the development of a prolonged discharge. Such a repetitive response to a motor nerve volley by a muscle fiber

during eserine perfusion has been observed in mammalian muscle but not in frog muscle (78, 80). There is also some earlier evidence [cf. Brown (73)] that small concentrations of eserine increase the discharge from the cervical sympathetic ganglion. Eccles (62) on the other hand, does not accept these facts as evidence for a prolonged life of the transmitter. In the case of muscle he assumes that the eserine makes the muscle fibers hyperexcitable so that they are set into prolonged rhythmic activity by a single motor nerve volley. He further supports his view by pointing out that the "detonator response" is not lengthened by eserine (67) and that eserine usually fails to produce a marked after-discharge from a sympathetic ganglion (48, 81). It is generally admitted that the effects of eserine are uncertain and not understood (73). This is in part due to the fact that it probably has specific actions other than that of protecting acetylcholine. And there is the further complication that when acetylcholine is permitted to reach a high concentration it depresses the activity of nerve and muscle cells. In view of such considerations we do not feel that the uncertain action of eserine should be considered strong evidence against the neuro-humoral hypothesis.

A third explanation of synaptic transmission is more inclusive than either of those described in the preceding paragraphs, but is in conflict with neither of them (82). It recognizes that any change in the environment of a nerve cell will necessarily alter the basic structure of the cell and will, accordingly, tend either to excite or depress its activity. Transmission across a synapse would consequently be accomplished by the net effect of all the chemical, electrical and resulting ionic changes which are produced by the presynaptic impulse, and would also be determined by the accumulated effects of previous activity. In agreement with such an explanation are the observations that during perfusion of a ganglion with acetylcholine submaximal volleys of preganglionic impulses excite many cells which do not respond in the absence of the acetylcholine (48); that there is a similar potentiation of impulse transmission by certain alterations in the calcium and potassium concentrations in the perfusing fluid and depression by others (65); and that there is a direct excitation of the ganglion cells by shifts in the concentration of these ions (65). And, furthermore, Larrabee & Bronk (83) have recently reported that there is sustained discharge from a ganglion many seconds after the end of a pregan-

glionic titanus. This forces us to conclude either that acetylcholine is not present as an active stimulant only during the presynaptic spike potential (74) or that there are other accompaniments and results of the nerve impulse than acetylcholine or the action potential which play a part in effecting synaptic transmission. Some of these must persist for a considerable time.

#### LONG-LASTING EFFECTS OF NERVOUS ACTIVITY

Eccles (84) has shown that some ganglion cells have an increased excitability for 200 msec. after a preganglionic volley. He refers to this state of increased excitability as central excitatory state (c.e.s.), but according to him it is not capable of initiating a discharge of impulses without the development of another "detonator response" by a subsequent presynaptic impulse (67). Eccles, therefore, defines the central excitatory state as an altered state of a nerve cell which is the result of previous activity and which, during its course of several hundred milliseconds, increases the excitability of the cell. This term, central excitatory state, should be used with understanding and caution and should not be assumed to indicate a knowledge of excitation processes which we do not now possess. It does not appear to be other than a measure of the excitability of a nerve cell. In using it one should bear in mind that the excitability of nerve cells is continually fluctuating and is modified by various changes in their environment.

In the stellate ganglion there have been observed (85, 86) periods of increased excitability which last for many seconds following a single submaximal volley. Because of this persisting condition, cells which were subliminally excited by the first volley will respond to a second similar volley even though it follows the first by five or more seconds. The degree and duration of facilitation is increased by repeated stimulation, for following a short tetanus the number of ganglion cells which can respond to a submaximal volley of given strength may be increased four to five fold. And not for sixty seconds will the excitability return to its pre-tetanus level. This is an excellent illustration of how the capacity of nerve cells to respond to nerve impulses is continually modified by the trains of impulses which constitute the messages of the nervous system.

Similar long-lasting augmentation of the response of normal and partially curarized muscle to a motor nerve volley has been

investigated recently. Guttman, Horton & Wilber (87) believe that the phenomenon in normal muscle is due to an accumulation of a chemical mediator such as acetylcholine or epinephrine at the junctional region. Rosenblueth & Morison (88), however, object to this interpretation on the grounds that such substances could not persist long enough to account for the prolonged augmentation, and suggest the mobilization and persistence of potassium ions as a more reasonable explanation. Still another view of the phenomenon is taken by Brown (89). Because he observes no increase in the action potential which accompanies the increased twitch following the tetanus he concludes that the latter is not due to a myoneural process at all, but to an increased capacity of the muscle fibers to develop tension. On the other hand, he finds that the potentiated response of a partially curarized muscle is due to the activity of more muscle fibers and is, therefore, the result of the prolonged persistence of a lowered threshold of the end-plates or of a more effective transmitting mechanism. This he ascribes to the effect of potassium ions because the injection of small concentrations of potassium chloride gives after-results which are similar to those following a tetanus. Why a sympathetic ganglion should resemble a partially curarized muscle as regards post-tetanic potentiation and whether it is indeed the same type of mechanism have not yet been determined. But we can say that perfusion of a ganglion with potassium chloride does increase the ganglionic discharge initiated by a preganglionic volley (65).

The magnitude and duration of such long persisting states of excitation, or changes in threshold, depend upon the frequency and duration of the trains of impulses. But inasmuch as they are developed to a small degree by a single impulse, they might be expected to modify the course of events associated with a low frequency tetanus. This is indeed the case, for impulses coming to a ganglion at rates of three to ten per second may cause a progressively increasing number of ganglion cells to respond (48). This recruitment of cells must be due to persistence of some effects of the preceding impulses so that the threshold of more and more cells is gradually attained. All of the conditions which favor such recruitment are not known. It certainly is marked in a ganglion with inadequate circulation for as the cells lose their capacity to respond to a single volley they require the summated effects of a train of impulses. Gasser (39) has found a similar recruitment of

axons in peripheral nerve trunks which is due to a long-persisting state of excitation in the nerve. This appears under certain conditions which also favor the development of large negative afterpotentials. Such a summation of excitation by infrequently spaced impulses probably underlies many of the phenomena of reflex action.



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ELDRIDGE REEVES JOHNSON FOUNDATION AND THE  
INSTITUTE OF NEUROLOGY  
UNIVERSITY OF PENNSYLVANIA  
PHILADELPHIA, PENNSYLVANIA

## THE AUTONOMIC NERVOUS SYSTEM\*

BY JOSEPH C. HINSEY

*Department of Physiology, Cornell University Medical School  
New York City*

This review deals with autonomic nervous system and some of the problems involved in its peripheral representation. While Langley used the term "autonomic" to apply to the efferent components, we shall use it here as synonymous with "visceral nervous system," which is an aggregation of ganglia, nerves and plexuses through which the viscera, glands, heart and blood vessels, and smooth muscle, wherever it occurs, receive their innervation (167). It has a sensory and a motor component, is under the control of integrating mechanisms in the central nervous system, influences and is influenced by the cerebrospinal nervous system. It will be necessary to omit a discussion of central integrating mechanisms at the spinal, bulbar, diencephalic and cortical levels in order to discuss briefly some of the publications which have appeared mainly in the last two years in other fields of interest. The voluminous literature pertaining to the autonomic nervous system has been dealt with in a number of monographs and textbooks (23, 119, 197, 203, 241, 318, 376).

### EMBRYOLOGY AND ANATOMY

Although Van Campenhout (69) and others believe that the sole origin of sympathetic ganglion cells is in the neural crest, Raven (304) and Jones (187) concluded from experiments with amphibians and chick embryos that sympathetic elements are derived not only from neural crest cells but also from the ventral portion of the neural tube. Detwiler (96, 97) stained the various embryonic cellular elements in amphibians with the Nile blue sulphate technique and showed that the cells which migrate from the neural crest to form the sympathetic ganglia move to their positions early in embryonic life. Therefore, it would be possible to remove the neural crest at such a time that the development of spinal ganglia would be prevented and yet would permit the formation of sympathetic elements.

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The dissections of the autonomic nervous system, which Zuckerman (390) has described in a large number of rhesus monkeys and other primates, are important because they show the variations which take place. They indicate the extreme care that must be exercised in the interpretation of experiments on these animals. For example, the caudal limit of emergence of white rami was the third lumbar root in 51, the fourth lumbar in 57 and the fifth lumbar in 3 specimens of rhesus monkeys. The sacral preganglionic fibers usually emerged in the first and second sacral segments and he suggested the possibility that some of these fibers passed rostrally along the hypogastric nerves. [See Trumble (364)]. Descriptions of the topography of the sympathetic trunk may be found in other papers (219, 220, 290, 294). Haggqvist (136) made an analysis of the fiber sizes of nerve fibers in the ventral roots of monkeys.

The innervation of the pupil was described by Foerster, Gagel & Mahoney (110) and the courses of the sympathetic and parasympathetic nerves in the orbit of the cat by Christensen (77). In following the development of the autonomic innervation of the fetal pig iris, Christensen (78) found that the sphincter iridis muscle was refractory to carbamylcholine until the nerve fibers grew into the iris; the responses were at a maximum in fetuses of 220 mm. Experimentally, Crouch (88) was able to show that the efferent fibers from one Edinger-Westphal nucleus pass into both oculomotor nerves. The most complete set of experiments on the innervation of the intrinsic eye-muscles was performed by Clark (79). In the cat, the ciliary muscle and the sphincter of the pupil are supplied by fibers arising in the ciliary ganglion and the dilator muscle by ones from cells in the superior cervical sympathetic ganglion. Each smooth muscle fiber was supplied by at least one termination and no intraprotoplasmic endings were seen.

Huyghebaert (180), Larsell, Barnes & Fenton (226) and Kuntz (204) have dealt with the innervation of the nasal mucous membrane. Kuntz believes that the nasal mucous membranes have a sensory innervation, the fibers of which arise from the dorsal root ganglia in the upper thoracic segments. Takino & Miyake's (348) description of the innervation of the fetal lung adds little, if anything, to our previous concepts.

An excellent experimental analysis of the vagus nerve has been made by DuBois & Foley (99) and Foley & DuBois (112). After

degeneration of the motor components, the number of nerve fibers proximal to the nodose ganglia was only 65 to 80 per cent of that distal. This means that there is a discrepancy in counting, that there are cells in the nodose ganglion without central processes, or there is a branching of some of the distal processes near their cells of origin. Their counts were done accurately on excellent preparations but their work is being continued in an endeavor to unravel this difficult problem. Jones (188) has made counts proximal and distal to the nodose ganglion and found an excess distally which he attributed to the possibility that a considerable number of motor fibers arise from cells in the nodose ganglion. It is rather generally admitted that there are no synapses about the unipolar ganglion cells in the nodose ganglion (303). Heinbecker & O'Leary (145) described motor effects in the lungs and duodenum on stimulation of fibers arising in the nodose ganglion but they failed to find any effect on the heart (306). The presence of sensory neurons in the vagus below the nodose down as far as the thorax has been reported (59).

Different nerves to the heart have been sectioned and degenerated and the remaining terminations have been studied (279, 297, 319). Maksudowa (259) stained the terminations of the vagus about the ganglion cells in the wall of the heart. When Puddu (300) and Jourdan & Froment (190) sectioned the atrio-ventricular bundle, stimulation of the left vagus still slowed the rate of the dog's ventricle. This may be attributed to liberation in the region of the atrium of vagus stuff which passes to the ventricle or there must be post-ganglionic neurons under vagal control which course directly to the ventricle. The neuro-anatomical explanation of this phenomenon is not entirely clear. Studies on the accelerator pathways have been made (131, 278). After section of the atrio-ventricular bundle, Jourdan & Froment (191) found that stimulation of the stellate ganglion produced an increase in frequency of the ventricular contractions as well as those of the atria, an experiment which shows that either postganglionic sympathetic fibers pass outside the atrio-ventricular bundle or the production of sympathin is responsible for the increased frequency of ventricular contractions.

Goormaghtigh (128, 129) found ganglion cells situated between the diaphragm and the coeliac ganglion in the right vagus. These were called abdominal vagal paraganglia; their preganglionic fibers

were thought to be from the vagus but their terminations were not described. According to Jemerin & Hollander (185), both the anterior and posterior gastric vagi pass along the lesser curvature and send branches toward the greater curvature. In the customary incisions used in the preparation of Pavlov pouches, a large number of the vagus fibers would be sectioned.

Ottoviani (292) has described the innervation of the mammalian esophagus, Stöhr (343) that of the gastric glands and Nolf (281) that of the gastro-intestinal tract. Young's (387) account of the innervation of the viscera of the teleostean fishes is interesting in that he reports no sacral parasympathetic system. The intramural innervation of the gallbladder has been investigated by Stefanescu & Bratianu (336), Van Campenhout & Grenade (70) and Sabussow & Ssuslikow (315); and Jayle (184) studied the nerve supply of the liver.

Hollinshead (175) and Swinyard (347) made experimental morphological investigations upon the innervation of the adrenal medulla, the embryologic derivation of which would suggest a preganglionic innervation. After section of the splanchnics and removal of the lumbar sympathetic chain, Hollinshead found the only fibers remaining, after appropriate degeneration intervals, were apparently distributed to blood vessels. The absence of an innervation to the cortex and the fact that preganglionic fibers terminated among the chromaffin cells of the medulla was emphasized. After cutting and allowing the preganglionic fibers in the splanchnic to degenerate, Hermann & Jourdan (149) stimulated the splanchnic and showed by a cross circulation experiment that no epinephrine was produced. This supports the anatomical work which shows a preganglionic supply to the adrenal medulla. Of course, the postganglionic neurons might escape being activated during stimulation of the splanchnics if they were situated nearby the chromaffin cells. After degeneration procedures, Hollinshead (176) found that abdominal chromaffin tissue is also innervated by preganglionic fibers. Golube (127) studied the development of the adrenals and their innervation in the chick and Lawton (231) the nerve supply to the adrenal of the alligator.

Hermann & Jourdan (147, 148) sectioned the splanchnic nerve in the thorax of dogs and resected the sympathetic chain from the ninth through the thirteenth thoracic ganglia. After degeneration, stimulation of the splanchnic in the abdomen produced no evi-



dence of epinephrine secretion, but on stimulation below the renal ganglion, constriction occurred in the renal blood vessels. After degeneration of nerves from the splanchnics, the upper lumbar sympathetic trunks, and vagus, Kuntz (205) observed that many synapses and terminal branches of axons are left intact in the coeliac ganglia. Kuntz believes these remaining fibers and synapses are connected with nerve fibers arising in the enteric plexuses in the stomach and intestine and constitute reflex connections over which some gastro-intestinal reflexes are mediated. After nicotine had been injected over a period of several weeks into rabbits, Ingersoll (181) obtained evidence of cellular changes in the coeliac ganglia. This indicated to him that there had been increased functional activity of these postganglionic neurons.

Kuntz & Moseley (208) have described the following distribution of preganglionic fibers to pelvic ganglia in the cat: sacral to intramural ganglia of rectum; thoracolumbar and sacral to those of urinary bladder; and mainly thoracolumbar to ganglion cells in utero-vaginal plexus. This means that the nerve cells in pelvic autonomic ganglia may be postganglionic sympathetic or parasympathetic neurons and is in line with earlier work. Numerous papers dealing with the innervation of pelvic viscera have appeared (15, 63, 106, 125, 201, 253, 275, 301, 372, 374).

Kuntz, Alexander & Furcolo (207) present evidence from degeneration procedures in the cat that there are preganglionic fibers from the first, second and third thoracic segments which terminate in the inferior cervical ganglion. However, it must be shown conclusively that impulses over preganglionic fibers in the first thoracic ventral root exert an influence over the visceral structures in the upper extremity before conclusions can be drawn as to their significance in the sympathetic denervations of the arm. Fulton (119 p. 218) points out that, "the chief vasoconstrictor preganglionic control in man and monkeys comes from the thoracic levels 2, 3, 4, 5, and 6, and occasionally, from somewhat lower segments." In unpublished experiments from our laboratory, Hare has found that preganglionics in the first thoracic segments play no role in the control of visceral structures in the pads of the forelimbs of cats.

Physiological importance should be attached to a number of other morphological investigations (48, 55, 81, 137, 179, 265, 375). A comparison of visceral nerves of man with those of common

laboratory animals shows no significant variations in myelination (144).

During recent years, work has appeared which challenges our fundamental conceptions of the architecture of the autonomic nervous system. Stöhr (342, 343, 345) denies that sympathetic ganglion cells are separate individuals, and that they have processes which end freely, or in synaptic junctions. He contends that they form a syncytium throughout the entire body, and even goes so far as to deny the occurrence of nerve endings. Boeke (40) has described a sympathetic ground plexus, consisting of delicate interwoven and anastomosing nerve fibers running in strands or flattened bands with scattered nuclei, forming the ends of the sympathetic plexuses. This ground plexus is abundant everywhere in the smooth muscle tissues, glands, connective tissue and between skeletal muscle fibers, and it constitutes, in his opinion, the real efferent sympathetic terminal system. However, Boeke (41) admits the necessity for synaptic junctions everywhere in the end formation of the nervous system.

The validity of these concepts has been challenged (2, 156, 164, 167, 268, 277). However, a most complete critique of them has been made by Nonidez (282, 283, 284), who says, "In my opinion these reticulated formations consist of anastomosed argyrophil connective tissue fibers, stained with the ammoniacal silver solution of the method of Bielschowsky, for they do not appear in preparations with the reduced silver nitrate methods." Both Stöhr (343) and Boeke (41) have endeavored to answer Nonidez by claiming that his staining was insufficient. Nonidez has shown that he could duplicate their results with silver carbonate methods which are specific for reticular connective tissue. Their proof that the argyrophilic fibers, they demonstrate, are nerves is far from complete. Even Boeke's (41) claim that methylene blue demonstrates them is still fraught with the possibility, known to everyone who has worked with this stain, that sufficient oxidation will stain connective as well as nervous tissue.

The problem of the position of the terminal endings of visceral nerves has been discussed by Cannon & Rosenblueth (72) who have given the evidence for hypolemmal endings in smooth and cardiac muscle. Neuro-histologists are far from agreed that this type of nerve ending is at all common. In their methylene blue preparations, Kleynjens & Langworthy (201) observed that the

simple motor endings upon the smooth muscle fibers of the urinary bladder all have the same general appearance and are very numerous. Observations like these lead one to doubt the paucity of smooth muscle endings that has been described in a great many visceral structures of the body. The histology of nerve endings in smooth muscle must be put on a much firmer foundation before one can speak with any certainty as to intra- and extra-cellular formation of chemical mediators. The stumbling block is the great difficulty of obtaining satisfactory staining of nerve fibers in glands and in smooth and cardiac muscle.

#### VISCERAL AFFERENT PATHWAYS

The anatomical work which has been done to unravel the sensory pathways from the viscera has been reviewed recently (167). Like somatic afferent fibers, visceral afferents arise in dorsal root and cranial ganglia, their peripheral processes terminate in specialized receptors or free endings, they are myelinated or unmyelinated, and their central processes enter the spinal cord or brain stem. Aside from the fact that there are differences in their peripheral and central distributions, it seems doubtful if visceral afferent fibers should be assigned any fundamental properties differing from those of the somatic afferents. Their impulses may produce responses in somatic as well as visceral effectors, just as somatic afferent impulses may elicit activity in visceral as well as somatic effectors. Work dealing with the irradiation of autonomic reflexes has been chronicled by Schweitzer (322) in a monograph, in which a large amount of literature is reviewed in a manner which fails to present both sides of controversial subjects. Without any mention of a large body of evidence to the contrary, Schweitzer presents the "spinal parasympathetic" of Kuré as well established.

The visceral receptors in the carotid sinus and carotid body are still the subjects of intensive study. The carotid sinus has been assigned (a) reflex function in blood formation by Latner (228). In the absence of the innervation of the carotid sinus and aortic arch, labyrinthine stimulation produces a fall in blood pressure instead of a rise which is seen in normal rabbits on similar stimulation according to Mies (269). Démétriades & Spiegel (95) attribute the rise in blood pressure to the morphine anesthesia used in Mies' experiments. That cooling the nerve centers intensifies the inhibition of the heart produced by stimulation of the carotid sinus

nerve is reported by Tournade and Bernot (356). Ferris, Capps & Weiss (109) have studied the relation of the carotid sinus to the autonomic nervous system and neuroses. When pressor responses were elicited by temporary occlusion of the common carotid arteries in guinea pigs, three of eleven exhibited inhibition of intestinal peristalsis (183). Boyd & McCullagh (49) report that denervation of the carotid sinus and the aortic arch in the rabbit produces hypertension which does not persist in most cases. After chronic hypertension was produced in dogs by clamping the renal arteries, Bouckaert, Elaut & Heymans (44) found increased reflex vasoconstriction was caused by decreasing the intrasinus pressure. Furthermore, a pressor response produced by asphyxia to the vasoconstrictor center is accompanied by vasoconstriction in a skinned limb, while the rise in blood pressure brought on by reduction of the pressure in the carotid sinus is accompanied either by a dilatation or no volume change in a skinned limb (252).

The circulatory changes produced by stimulation of the pressoreceptors in the carotid sinus have been studied (80, 178, 380). Heymans, Bouckaert, Farber & Hsu (158) were able to stimulate the receptors in the carotid sinus with acetylcholine and induce vasomotor, cardio-inhibitory and respiratory reflexes. Bernthal (33) concluded that the carotid body is influenced by the carbon dioxide and oxygen tensions in arterial blood and is the source of a tonic chemo-reflex vasoconstrictor influence. However, it should be recalled that, in the excellent observations made by Bronk & Stella (51) on the responses in single end-organs in the carotid sinus, the pressure receptors were insensitive to the carbon dioxide and oxygen content of the blood.

Abe (1) has studied some of the efferent paths of circulatory reflexes elicited by stimulating the carotid sinus and aortic nerves in rabbits. When he stimulated the carotid sinus nerve before and after bilateral vagotomy, the fall in blood pressure was quite similar in both instances but the reduction in heart rate was less after the vagi were sectioned. In the dog, Hermann, Jourdan & Morin (150) sectioned both vagi and then destroyed different portions of the thoracic spinal cord. On stimulation of the nerve to the carotid sinus, a fall in blood pressure was present until the first thoracic segment was destroyed. This observation is important because there was no detectable fall in blood pressure due to

impulses travelling out over the cranial and cervical nerves of the dog. The circulatory responses from carotid sinus stimulations will be considered again in the discussion of the completely sympathectomized animals.

The roles of the receptors in the carotid sinus and carotid body in the control of respiration are dealt with in several papers (84, 85, 105, 189, 333, 339, 340, 369, 370). Kaufman (198) stimulated receptors in the carotid body of the dog with sodium cyanide and excited respiratory reflexes. There was an associated inhibition of reflex contractions in the tibialis anticus muscle which he tentatively explained as due to descending inhibition of motor neurons innervating this muscle. This phenomenon, which is similar to ones presented in earlier literature, is important because it shows how visceral afferent impulses may have a profound influence on distant portions of the central nervous system.

Other papers deal with visceral afferent mechanisms influencing respiration, (6, 53, 124, 162, 263, 307, 308, 309, 310, 337, 351) and with the reflex control of the diaphragm (75, 314, 371). Thornton (354) found that stimulation of the phrenic nerve in the cat produced broncho-dilatation, the afferent pathway of which is in the phrenic and the efferent one in the vagi.

The influence of afferent impulses from various portions of the respiratory tract upon the vasomotor apparatus has been studied (42, 92, 154, 226, 321). Bolton and his coworkers have shown that, when deep breathing occurs in man, there is a diminution of finger volume. They think this is due to afferent impulses coursing over intercostal nerves. In his discussion of pleural shock, Capps (74) concludes that in most cases it is due to irritation of receptors in the parietal pleura and the subsequent depressor reflex, and only exceptionally to air embolism.

The significance of the carotid sinus and other afferent mechanisms in the control of cerebral circulation has been considered (47, 111, 114, 115). Bouckaert & Jourdan (47) observed about the same small amount of dilation of cerebral vessels following sympathetic denervation as before sympathectomy when the pressure within the sinus was reduced. Forbes & Cobb (113) have discussed the problem of the vasomotor regulation in the light of various experimental procedures and have emphasized the agreement that a constrictor and dilator control exists. However, Wiggers (378) has questioned the validity of the evidence for cerebral vasodilators.

The vasoconstrictor pathways to the cerebral vessels have been investigated (46, 60, 352). The preganglionic fibers pass in the first four thoracic ventral roots and the postganglionic ones along the internal carotid plexus.

Various reflex functions of afferent components in the vagus have been investigated: aortic nerve (141, 142, 173); depressor nerve (260, 311, 338); pressor afferents (146, 159); and afferents from ductus arteriosus (22, 349). Anatomical studies have been made on the distribution of receptors to the heart and great vessels (279, 298, 325). One of the finest series of anatomical papers is that of Nonidez (285, 286, 287), who, by using the Cajal technique and serial sections in young rabbits, cats and dogs, has reconstructed the distribution of the aortic nerves and the contributions to the aortic glomus. The latter resembles the carotid body and Nonidez has suggested it contains chemoreceptors (83, 369). Nonidez (288) describes receptors in the venae cavae and pulmonary veins which may very well be those stimulated in the Bainbridge reflex.

There are receptors located in the region of the vessels of the mesentery and of the thoracic aorta which modify blood pressure particularly through vasomotor mechanisms in the spleen, kidney and posterior extremities (157). The voluntary muscles are said to have receptors which are stimulated by muscular activity and raise the blood pressure and accelerate the heart rate (4, 5). Truex (363) has described terminations on peripheral vessels and Tournade & Goinard (362) have confirmed earlier work demonstrating sensory fibers carrying pain impulses from the distal portion of the hind limb of the dog course with the cerebrospinal nerves and are not broken by abdominal sympathectomy. In a large series of papers, Malméjac and his coworkers have described a peripheral reflex responsible for the dilatation of vessels which follows arterial occlusion. Certain cellular elements have been suggested as responsible (261) and the response is said to be extremely localized (262). However, there are no known nerve cells in relation to peripheral vessels in mammals.

The effect of stimulation of temperature receptors upon the reflex control of circulation has been studied (31, 160, 334, 365, 366, 367, 368). Pin prick of the legs of humans causes reflex vasoconstriction of toes, a response more easily obtained in the toes than in the fingers (98).

The Pacinian corpuscles serve as receptors stimulated by changes in state of the mesenteric vasculature (121; see also, 157). Digital compression of the urinary bladder and of the large intestine and the passage of feces into the rectum of the decerebrate cat bring about viscerosomatic reflexes whose effectors are skeletal muscles (21). Irving, McSwiney & Suffolk (182) have used dilatation of the pupil in cats under chloralose anesthesia and reflex changes of blood pressure in spinal and decerebrate cats as indices of visceral afferent impulses. Afferent pathways in the vagi and splanchnic nerves were traced from the stomach and duodenum, while they were mainly in the splanchnics from the jejunum and ileum. Stimulation of afferent fibers in the splanchnic nerves causes reflex contraction of the terminal small intestine and ileocaecal valve (33) and rises in the arterial, venous and cerebrospinal fluid pressures (243). Radványi & Gellért (302) applied electrical stimulation to various abdominal viscera and the coeliac ganglia and observed changes in the electrocardiogram in dogs. Distension of an intestinal loop causes inhibition of the spontaneous contractions of the stomach (221). Mechanical stimulation of the anal canal and rectum in the normal conscious dog inhibits the motility and decreases the tonus of the stomach and jejunal fistulae. This was absent when the splanchnics were sectioned and lumbar sympathectomy performed (386). The inhibition of denervated fistulae was present after vagotomy and removal of the medullary tissue of the adrenal due, they thought, to the liberation of an inhibitory substance at the abdominal sympathetic endings. Shay, Gershon-Cohen & Fels (326) stimulated the duodenum in man and report an inhibition of the hyperglycemia after oral administration of glucose. Ashkenaz (13), using the visceropanicular reflex, and McSwiney & Suffolk (254) the pupillo-dilator reflex as indices of visceral afferent conduction to the cord, have traced the segments at which sensory fibers in the splanchnic nerves enter the spinal cord.

Stimulation of the common bile duct in man by faradization (389) or by distension with saline (32) produces epigastric pain and back pain in the subscapular region. Ascroft & Davies (12) were unable to produce signs of acute intestinal obstruction when they mechanically distended intestinal loops of the cat over rather long periods of time. Stern (341) has described a case in which there were attacks of widespread autonomic discharges including



urination and defecation which were relieved by denervation of the right carotid sinus.

The effect on the vascular supply of the kidney has been studied after injection of cold Ringer's solution into the small intestine (82), and after stimulation of the carotid sinus (289) and of afferent fibers in the splanchnic nerve (8). In addition to the direct nervous influences on kidney vessels produced by stimulation of afferent nerves, Alekseeva & Babsky (7) present evidence based on work with cross-circulations and transplantations in dogs, which they believe supports a humoral control.

Reynolds & Kaminester (305) worked out the motor innervation to the rabbit's uterus and Labate & Reynolds (218) described the afferent pathways from the ovarian plexus of the cat. The latter follow closely the ovarian artery and do not course in the superior hypogastric plexus, the inferior mesenteric ganglia and the lower aortic plexus. In his work on the various possible afferent mechanisms involved in the production of ovulation in the rabbit, Brooks (54) concluded that sexual excitement rather than a specific group of afferent mechanisms called forth ovulation in this form.

Reflexes involving responses in the eye have been examined in the light of the identification of afferent pathways. In a study of simultaneous reactions of different autonomic indicators, Darrow and Gellhorn (93a) found that stimulation of nerves like the sciatic and femoral caused dilatation of sympathectomized and normal pupils when reactions of other autonomic indicators were delayed or lacking. Magoun, Atlas, Hare & Ranson (257) have shown that the afferent fibers from the optic tract (monkey) which cause the pupillary light reflex pass to the pretectal region rather than to the superior colliculus as it is illustrated in some of the recent textbooks. Acheson, Rosenblueth & Partington (3) produced contraction of the cat's nictitating membrane by stimulation of a number of somatic and visceral nerves. The hamstring and saphenous afferents exerted a greater effect on the nictitating membrane than the hypogastric ones, while the reverse was true as far as pressor effects were concerned. The hypoglossal nerves contained afferent fibers having the capacity to produce visceral reflexes. Six to twenty days after removal of the superior cervical sympathetic ganglion, stimulation of somatic afferent fibers produces contraction of the denervated nictitating membrane due to reflex liberation of sympathin (240). By using spinal cord lesions

in cats (chloralose anesthesia), Harper & Swiney (139) have traced the ascending pathways traversed by impulses from somatic and visceral afferent nerves which produce pupillary dilatation. They concluded the central pathways for both appeared to be identical and were found in the lateral columns of the ipsilateral and contralateral white matter of the spinal cord. Marquis & Williams (264) have examined a series of human cases with central nervous system lesions. Their evidence prompted them to make the suggestion that vasomotor reactions incident to somatic painful stimulation were produced by impulses which ascended via the spinothalamic tracts.

The pathways from and to the urinary bladder have been exhaustively studied by Langworthy and his coworkers (201, 224, 225, 233). There are complicated arborizations upon the smooth muscle of the bladder which resemble the smooth muscle spindles present in other organs (201). These are supplied by sensory fibers entering the spinal cord in the sacral segments and respond to stretch. Pain impulses traverse afferent fibers in the hypogastric nerves. Talaat (350) has stimulated receptors in the urinary bladder and urethra and recorded potentials from the hypogastric, pelvic and pudic nerves in dogs. In Watkins' (373) experiments, distension of the urinary bladder raised blood pressure but the nictitating membrane either contracted or relaxed.

In addition to their importance for reflex responses, visceral afferent pathways are of great interest because of the part they play in visceral and referred pain. Many interesting papers have appeared recently (35, 39, 267, 273, 274, 280). Leriche's monograph (232) is a record of his large experience on the surgical relief of somatic and visceral pain. Moore (270) has reviewed the experimental work which he and his associates have performed on the types of stimuli producing visceral pain in the experimental animals and the pathways traversed. These experiments, which are too extensive to discuss here, represent some of the most carefully controlled ones which have been done.

Somatic pain may serve as the afferent stimulus to produce a peripheral vasodilatation, and, according to Stabins, Thornton & Scott (335), the efferent side of the reflex may not be limited to the peripheral nerve distribution in which the pain originates. They described two cases with glomus tumors which were surgically removed. The fact that the vasodilatation remained two or

three months postoperatively, when there was a painful scar, suggested to them that pain arising in the scar served as the afferent stimulus to the reflex arc.

Livingston (242) has reported ten cases of disability caused by pain in the extremities following trivial injuries. In addition to pain reactions which appeared excessive, there were vasomotor phenomena, hyperesthesia, referred pains and trophic changes. These post-traumatic pain syndromes were treated by blocking afferent impulses from the peripheral "trigger" mechanism or by blocking the efferent pathways in the thoracolumbar outflow. Livingston suggests the probability that tissue injury may start a cumulative process which is not limited to a single nerve distribution and which spreads and brings about reflexes involving the sympathetic nerves.

The clinical observations of Lewis (234) on the hyperalgesia in an area about a crushed portion of skin or a point of faradic stimulation led him to claim that there is a "nocifensor" system of nerve fibers of dorsal root ganglion origin that should be differentiated from sensory and sympathetic fibers. His argument depends upon the assumption that cutaneous localization depends upon a single sensory fiber to each receptor rather than on patterns of impulses over several fibers, each of which may branch considerably.

In a study of visceral and referred pain, Pollock & Davis (299) have stimulated the central portion of the diaphragmatic peritoneum and followed the effect of various lesions upon pain responses in unanesthetized animals. Among other things, their experiments indicated to them that the pain accompanying stimulation of the central diaphragmatic peritoneum was not present when the sympathetic pathways to the cervical distribution were interrupted. They suggest that sympathetic impulses to the periphery bring about some vasomotor or hormonal changes which in turn stimulate cerebrospinal endings. The resulting impulses would travel back to the spinal cord and account for the peripheral reference. This gives support to the viscerocutaneous reflexes as the explanation of referred pain. However, Hinsey & Phillips (171) elicited pain responses on stimulation of the central portion of the diaphragmatic peritoneum of unanesthetized cats in which the sympathetic chains had been removed bilaterally from above the superior cervical ganglia to below the eighth thoracic segment in addition to spinal cord section in the upper thoracic region. Un-

published experiments on dogs show the same to be true after bilateral removal of the stellate ganglia and spinal cord section at the seventh cervical segment. While pain responses are still present after appropriate sympathectomy in animals, it cannot be concluded that viscerocutaneous reflexes play no part in referred pain in man.

Morley (273) has built up a case for antidromic dorsal impulses being responsible for peripheral reference, one which is far too premature to evaluate on the basis of present evidence. Just as visceral afferent impulses may inhibit or facilitate muscular reflexes (102, 198, 323), they may very well change the threshold in the spinal cord to afferent impulses from the periphery. In other words, it does not seem that present information permits a complete discarding of the "hyperirritable focus" concept of the older literature in the explanation of referred pain.

#### REFLEXES IN AUTONOMIC GANGLIA

The question of whether there are reflexes in the chain and prevertebral ganglia of the thoracolumbar division of the autonomic nervous system is an old one, which, apart from the large number of observations on axon reflexes, has been answered essentially in the negative fashion. However, the work of Schwartz (320), which has been accepted in many quarters, indicated the presence of such reflexes involving synapses in the extraspinal sympathetic ganglia. Schwartz endeavored to isolate the stellate ganglia from connections with the central nervous system by sectioning the afferent and preganglionic supply to the upper extremity in the cat. After this, testing with the skin galvanic reflex technique was said to show the presence of reflexes. However, in Hare's (138) analysis, it was shown that if all the sensory pathways to the upper extremity of the cat were cut (dorsal roots from the third cervical down through the fifth thoracic) and the preganglionic fibers cut by section of the thoracic ventral roots from the eighth cervical down through the tenth thoracic, no evidence whatsoever was found to support Schwartz's findings. That Hare's technique was adequate was shown by control observations.

Bolton, Williams & Carmichael (43) have reported two human cases of spinal cord lesions in which the posterior root and sympathetic ganglia were intact. None of their observations in the lower extremities indicate reflex vasomotor responses mediated through

the sympathetic chain ganglia. Govaerts (130) described axon-reflexes through the cardiac branches of the stellate ganglion of the cat but he (131) states that the isolated stellate ganglion has no reflex activity. Likewise, Bronk, Tower, Solandt & Larrabee (52) could not find ganglionic reflexes. Following destruction of the thoracolumbar spinal cord in dogs, Hermann, Morin & Cier (153) deny that the sympathetic chain ganglia exert any reflex control of the vascular bed in the hind limbs. Lewis & Palser (235) attribute the fall in blood pressure during spinal anesthesia in man to vasomotor paralysis, one which would be brought about by narcotizing the preganglionic fibers. In their observations, peripheral reflexes, if present, were not sufficient to maintain the blood pressure. Bradshaw (50) has studied the effect of spinal anesthesia in animals and reports a fall in blood pressure in normal cats (anesthetized with sodium barbital) and the absence of a fall in completely sympathectomized ones under similar conditions.

Alvarez (9) has used anoxemia as a means of analyzing the nervous control of the small intestine of the rabbit. Dechaume (94) destroyed the spinal cords of six dogs below the first thoracic segment. During a period of 15 to 190 days, no evidence of dysfunction of the autonomic nervous system was seen even in one which also had intrathoracic section of both vagi. This was attributed to peripheral mechanisms functioning without connection with the central nervous system. Hermann, Morin, Jourdan & Vial (155) found that, after destruction of the spinal cords below the cervical region in dogs, the digestive functions were carried on quite well. However, in one dog in which the vagi were also sectioned, there were variations in appetite, loss of weight and a gastric ulcer was found at autopsy. Lawson & Holt (230) believe that the decentralized inferior mesenteric ganglion in the dog exerts an effect upon the function of the anal canal and distal colon. Such experiments, however, are fraught with the difficulty of removal of all of the preganglionic fibers and the possibility of interfering with the arterial blood supply in the inferior mesenteric artery at the time of operation.

As has been discussed earlier, Kuntz (205, 206) has described synapses in the celiac ganglion, the terminal axons of which are said to be connected with nerve fibers arising in the enteric plexuses in the stomach and intestine. The celiac ganglion is a difficult one to approach experimentally because of the difficulty of being

certain that a few preganglionic fibers are not missed. The possibility of regeneration here is very good. Furthermore, such synapses have been reported absent in the chain ganglia such as the sacral ones in well-controlled observations. Such synapses as Kuntz has described cannot be considered as thoroughly established.

#### CHEMICAL TRANSMISSION OF IMPULSES

The problem of chemical transmission, which was approached first in relation to the postganglionic neurons and their terminations, has been extended to the transmission at the terminations of the preganglionic visceral neurons and somatic motor neurons, and finally to the synapses in the central nervous system. The reviews dealing with this problem have been extensive (14, 16, 58, 67, 72, 89, 90, 91, 103, 104, 145a, 244, 245, 312). The terms sympathetic and parasympathetic as applied to the autonomic nervous system must be used on an anatomical rather than a functional basis. The terms that have been used for a functional classification are cholinergic and adrenergic (72, p. 31). The postganglionic fibers in the sympathetic outflow may be adrenergic or cholinergic; those in the parasympathetic, cholinergic and possibly adrenergic. The preganglionic fibers in both divisions are said to be cholinergic as are the somatic motor ones. Some of the fibers in posterior roots are cholinergic, that is, the ones which produce vasodilatation (384).

The pendulum has swung completely in the last ten years toward chemical transmission. Although the evidence at times seems overwhelming in favor of acetylcholine as a synaptic transmitter, what seems to be a tenable position is expressed by Fulton (119, p. 75), "Synaptic transmission is probably brought about directly by the action currents of the axon terminal rather than by a specific humoral agent such as acetylcholine, since the time relations of synaptic transmission are not altered by agents which affect the action of these chemical substances (Eccles). Acetylcholine appears to be a by-product of nerve metabolism but its presence affects the resting threshold of neurons (c.e.s.)." Gasser (122) presents the case for the action potential, which certainly cannot be eliminated as a synaptic transmitter.

That postganglionic neurons produce chemical substances at their terminations, sympathin in some and acetylcholine in others, seems to be established. The reviews which have been cited give the evidence supporting this and only a few of the many recent

papers can be cited here. Magoun, Ranson & Hetherington (258) have shown that stimulation of the hypothalamus in the cat causes the liberation of epinephrine and sympathin. Hermann, Jourdan, Morin & Vial (151) likewise have demonstrated sympathin production in the dog by stimulation of the central nervous system as well as the splanchnic nerve in the absence of the adrenal medulla. On the other hand, Gayet, Minz & Quivy (123) state that acetylcholine is present in the venous blood of the stomach during stimulation of the splanchnic nerves.

Ross (313) has studied the effect produced by the injection of substance 933F (piperidinomethylbenzodioxane) upon the responses in pregnant and non-pregnant cat uterus when the hypogastric nerves were stimulated and when epinephrine was injected. Kennard (199) followed the effect of progestin on the non-pregnant cat uterus in relation to responses to epinephrine and nerve stimulation. Morison & Lissák (272) studied the influence substance 933F exerts on responses in the nictitating membrane and vascular system. The breakdown of epinephrine was accelerated *in vitro* but they argue against a change in cellular permeability. In the nictitating membrane of the cat, epinephrine and acetylcholine are said to produce similar responses, ones that may be summed one with the other when both are given together (271). In the frog's heart, Lissák (238) found that calcium causes the production of a substance which closely resembles sympathin. Greer, Pinkston, Baxter & Brannon (134) believe that there are at least two adrenergic mediators: "Sc" which resembles *l*-nor-epinephrine and has a greater intrinsic power of inducing contraction and less intrinsic power of inducing relaxation than "Sr" which resembles *l*-epinephrine. It is suggested that both of these substances are liberated by postganglionic sympathetic fibers. This does not necessitate the intervention of cellular constituents as is the case in the explanation of the two sympathins, E and I, of Cannon and Rosenblueth. Blaschko, Richter & Schlossmann (38) have studied the inactivation of epinephrine produced by an oxidase present in liver extracts, one of which differed from catechol oxidase. Bacq & Heirman (20) contend that the inhibitory action of epinephrine appears to be a product of a phenolase acting upon epinephrine to produce adrenoxine, an action taking place within the smooth muscle cells. This conclusion does not apply to the action of epinephrine upon the intestine.



Contrary to their earlier observations and in keeping with those of Hermann, Jourdan, Morin & Vial (152), Cannon & Rosenblueth (73) have found that a small amount of epinephrine can be liberated by strong electrical stimulation of the denervated adrenal medulla of the cat. This shows that the medullary cells can be activated by changes of potential when the preganglionic neurons are absent. Consequently bioelectric potentials cannot be completely ruled out in the activation of cells in the adrenal medulla.

Burn (66) reviewed the evidence bearing on the presence of sympathetic vasodilators, some of which are cholinergic and presumably some are adrenergic. It is pointed out that there are important species variations which must be taken into account in the interpretation of experimental work, *i.e.*, there are sympathetic vasodilators in the skeletal muscles of the dog and hare, few in the cat, and none in the rabbit and monkey. There is none in the skin of the dog with the exception of that in the ear. The sympathetic motor fibers to sweat glands are classified as cholinergic and are considered in papers by Darrow (93) and List & Peet (239).

Some of the pseudomotor contractures, which are present in mammalian skeletal muscles after section and degeneration of somatic motor nerves, are of interest in regard to sympathetic vasodilator nerves of the cholinergic variety. These denervated muscles fail to show contractures when epinephrine is injected but do so with acetylcholine and a number of other substances. Bülbring & Burn (64) confirmed and extended the work of Hinsey & Cutting (169) who first contended that the Sherrington phenomenon is a physiological artifact caused by a vasodilator substance (presumably acetylcholine), produced by postganglionic sympathetic fibers and acting upon sensitized denervated skeletal muscle. In their work in pseudomotor phenomena in the head region, Lewy, Groff & Grant (236, 237) conclude that the mesencephalic nucleus of the trigeminal nerves gives rise to autonomic fibers passing to muscles in the eyelids, face and tongue. Grant & Kirby (133) found that, on stimulation of the chorda tympani, the Vulpian-Heidenhain contracture was present in the tongues of both dogs and cats, but they could obtain it from stimulation of the cervical sympathetic trunk only in the cat. It is difficult to say whether this difference in cat and dog was due to a species difference in the distribution of cholinergic fibers or whether techniques

in experimental degenerations were responsible. Tournade & Chevillot (357, 358, 359, 360, 361) have studied the tongue phenomenon in some detail and report that acetylcholine was present in the eserinated venous blood from the tongue after stimulation of the chorda-lingual nerve.

During fright, Bender (29) observed contractures in denervated facial muscles which he attributed to a secretion of acetylcholine into the blood stream similar to that of epinephrine. This work has been discussed by Hinsey & Cattell (168). Bender & Kennard (30) have done more complete denervations by section of combinations of cranial nerves in addition to removal of the entire cervical sympathetic chains. The "fright reaction" was found to be present. It is amazing that such quantities of acetylcholine could get into the blood stream in the absence of eserine because the local destruction of acetylcholine by choline esterase is an important factor in the chemical transmission concept. Furthermore, one would expect to see evidence of other actions of acetylcholine if it were present in sufficient concentrations to cause contractures in denervated skeletal muscles. Direct assay of the blood for acetylcholine would be necessary to absolutely identify the contracture producing substance, as Wybauw (383) has done for venous blood from a cat's paw.

Jourdan & Nowak (192) are convinced that there are vagal fibers, originating in the dog's medulla oblongata, that accelerate the heart. If these fibers are present, it is tempting to suggest, as others have done, that they are adrenergic. Jung & Pierre (194) deny the existence of such fibers in the vagus of the pig and Kabat (195) failed to find them in the dog when he stimulated intracranial vagal rootlets.

According to Cannon & Rosenblueth (71), when preganglionic fibers supplying the superior cervical sympathetic ganglion were sectioned, the ganglion itself became sensitized to acetylcholine. This observation, says Brücke (61), receives adequate explanation from the fact that the smooth muscle of the nictitating membrane, which they had used to test the activity of the ganglion, was sensitized to epinephrine and sympathin. Brücke (62) made assays for choline esterase in the superior cervical and nodose ganglia and reported a concentration about three times greater in the former. This difference disappeared following section and degeneration of the preganglionic fibers. After section of the preganglionic fibers,

MacIntosh (250) found that failure to transmit impulses through the ganglion occurred at a time when most of the preformed acetylcholine disappeared.

Lorente de Nó (246) perfused the superior cervical ganglion of the cat according to the technique which Feldberg & Gaddum (108) had used. Acetylcholine was found in greatest amounts in the perfusate when the ganglion cells were injured, it appeared sometimes minutes after stimulation of preganglionic fibers, and it was present after stimulation of postganglionic fibers, contrary to the observations in Dale's laboratory. While admitting a metabolism of acetylcholine in the tissue of the ganglion, he does not consider this metabolism specific only to the synapses. MacIntosh (251) introduced modifications of the original perfusion technique, involving pulsatile pressures and a modified perfusion fluid (Locke's solution with a ten per cent dilution of heparinized cat's blood). He found that there was a close correspondence between the output of acetylcholine and the periods of stimulation of preganglionic fibers. Gaddum, Khayyal & Rydin (120) insist that acetylcholine is liberated by an isolated vagus nerve only when the stimulation is of such nature that the nerve is damaged.

Chemical transmission and nervous control in the salivary glands has been studied by a number of investigators (34, 200, 222, 223, 249, 324).

#### SENSITIZATION BY DENERVATION

The sensitization to different substances which follows denervation of smooth muscle is still under active investigation. Bacq (17, 18) found that a number of substances which inhibit the oxidation of epinephrine *in vitro* sensitize the smooth muscle of the nictitating membrane to this substance. Wolff & Cattell (381) suggest that in the absence of nerve impulses there is an accumulation of a substance (or a state) in the effector cell which increases its excitability; on the other hand, this factor is reduced when the nerves are stimulated. Heinbecker (143) followed the effect of denervation plus thyroidectomy, gonadectomy or adrenalectomy on the response to exogenous epinephrine. His evidence was compatible with the interpretation that increased sensitivity of smooth muscle to epinephrine may be caused by an increased store of epinephrine or of something upon which it acts in the smooth muscle cells. Simeone (328) has given the following possibilities

to explain sensitization after denervation: increased permeability of effector cells, impaired ability to destroy the mediator, increased store of mediator's precursor in cells, or altered physical properties of the contractile cells. Simeone (329) followed the sensitization to epinephrine after denervation of the cat's nictitating membrane and its disappearance concomitant with regeneration. Bülbring & Burn (65) emphasized that denervation sensitization to tyramine and epinephrine may be present with small doses but may be reversed with large concentrations. Denervation increases the inhibitory effect of epinephrine in a number of structures: cat bronchioles (87), rabbit intestine and cat uterus (248), and dog intestine (385).

Shen & Cannon (327) found a sensitization to acetylcholine in the denervated iris which appeared in twenty-four hours. Six and more days after section of the parasympathetic supply to the submaxillary gland in the dog, the gland shows a greater response to pilocarpine in the unanesthetized state (295). There was no increased response to acetylcholine after such denervation. Degeneration of the sympathetic innervation to the cat's submaxillary gland produces a sensitization to epinephrine and to pilocarpine but a less frequent one to acetylcholine (330). Such a denervation of the lachrymal gland produces a similar sensitization (255).

The problem of epinephrine sensitization is of importance in the interpretation of the return of abnormal vasoconstriction after postganglionectomy in vascular disorders in man. White, Okelberry & Whitelaw (377) present their case for sensitization to epinephrine and sympathomimetic substances as the cause of these returns of abnormal vasoconstriction. Ascroft (11) has attacked this problem experimentally in the monkey and his results are in line with conception of White and his coworkers. This concept has been challenged by Simmons & Sheehan (331) who interpreted the relapses which are more common in cervico-thoracic ganglionectomy as due to regeneration of sympathetic fibers and a return of nervous control. Fatherree & Allen (107) find that if normally innervated vessels are properly dilated, exogenous epinephrine in small doses causes marked vasoconstriction in the digital arterioles of normal patients. Grant's (132) experiments on the resumption of tonus in the ear vessels of rabbits support a sensitization to an epinephrine-like substance as the cause. However, this substance was present after adrenalectomy and hypophysectomy. It remains

to be seen whether or not the case for sensitization to an epinephrine-like substance in cases of removal of postganglionic neurons in man has been over-stated.

#### RESPONSES AFTER SYMPATHECTOMY

The responses to exercise and emotional excitement in the totally sympathectomized (all preganglionic thoracolumbar pathways interrupted) dog and cat have been observed in some detail. Pinkston, Partington & Rosenblueth (296) recorded reflex changes in blood pressure in such animals (cats and dogs) on stimulation of afferent fibers and found rises and falls. In cats, struggle produced a fall which was not present in dogs. This was the only difference in the reflex behavior in sympathectomized cats and dogs. They suggested that dorsal root dilators were involved in some of these extra-sympathetic pathways. However, Brouha, Cannon & Dill (56) found an acceleration in heart rate of the completely sympathectomized dog, both during excitement and exercise. In such a dog during exercise, the sugar and lactic acid levels in the blood, the alkali reserve and body temperature varied within normal limits. They attributed the cardiac acceleration to a reduction in the tonicity of the cardio-inhibitors of the vagi and to an increase in the tonicity of the vagal cardio-accelerators of the dog (192). Brouha, Dill & Nowak (57) could obtain this cardio-acceleration three weeks after sympathectomy in the dog, thus making regeneration of preganglionic fibers an unlikely cause for this type of response but not ruling it out completely. Bouckaert & Heymans (45) could not obtain similar results in the completely sympathectomized dog, where they believe cardio-accelerators in the vagus have no accelerating effect on the heart during rest or excitement. Hodes (174) has again repeated such experiments in sympathectomized cats with one adrenal removed. These animals could not withstand exercise as well as normal ones. Later as regeneration of the sympathetic fibers took place, there was a marked improvement in the ability to perform severe muscular exercise. His observations suggest the presence of cardio-accelerators in the vagus trunk which resist the action of atropine. It is important that the presence of such vagal accelerator pathways be established beyond peradventure, their presence be looked for in a number of different animals, and the possibility of sympathetic regeneration be completely ruled out. Here again such significant differences in these

two species show the danger of making generalizations from observations on one form.

Contrary to the observations of Bacq, Brouha & Heymans (19), Thomas & Brooks (353) found that total removal of both sympathetic chains in cats interrupted all important pathways of vasomotor reflexes brought about by pressure changes in the carotid sinus. These observations on cats were in agreement with those of Bacq and his coworkers (19) on dogs.

Wilson, Roome & Grimson (379) made a number of observations on sympathectomized dogs, among which was the fact that electrical stimulation of the central end of the cut sciatic nerve produced no effect upon the blood pressure in animals recently sympathectomized. In these animals with lowered blood pressures, the bleeding volumes were approximately normal. Grimson, Wilson & Phemister (135) followed blood pressures immediately and for some time after complete sympathectomy in dogs. Regeneration, a possibility which they admit, would seem to make their later observations difficult to interpret.

Freeman & Page (117) were able to produce hypertension by compression of the renal arteries in completely sympathectomized dogs and found that sympathectomy did not materially affect the level of blood pressure in animals in which hypertension had been induced previously. Hemorrhage does not produce shock in totally sympathectomized dogs in spite of the fact that they cannot withstand as much blood loss as normal animals and that their blood pressure goes to low levels (118). This was attributed to the fact that the tissues receive an adequate blood supply in the absence of the control of vasomotor nerves and epinephrine production. We have seen that Dechaume (94) and Hermann and his coworkers (155) destroyed the spinal cord below the cervical region to remove the activity of the thoracolumbar sympathetic division. Such animals differ from completely sympathectomized ones in that the sacral outflow is inactivated, the somatic motor fibers are degenerated in the area supplied by the destroyed spinal cord, and, of course, the sensory pathways from the periphery are broken.

#### REGENERATION IN AUTONOMIC NERVES

Experiments of morphological and physiological nature involving degeneration of autonomic nerves are fraught with the possibility of regeneration when carried over relatively long periods of

time. After anastomosing the vagus nerve with the peripheral nerve supply to the forelimbs of dogs, Anochin & Iwanow (10) studied the responses obtained by stimulating the skin of the extremities. Eventually, it was possible to restore normal function. In experiments like these, extreme care must be exercised to prevent regeneration of the normal supply to the part and to look for it with functional as well as anatomical methods at the termination of the experiment. De Castro (76) and Wolff, Hare & Cattell (382) have anastomosed cerebrospinal nerves like the hypoglossal and phrenic to the cervical sympathetic trunk in the neck. Fibers in these cerebrospinal nerves could act as preganglionic fibers to the superior cervical ganglion after regeneration had taken place. Morphological studies showed the finer relations of the regenerating fibers in the superior cervical ganglion. De Castro found that if sufficient concentrations of nicotine were applied, he could block transmission through the ganglion, where the terminations of the preganglionic fibers are larger than in a normal ganglion. The proximal portions of both the phrenic and hypoglossal nerves contain a component of postganglionic fibers. If these participate in the regeneration, it is possible that they might grow right through the ganglion to its peripheral distribution.

Lawrentjew & Borowskaja (229) have observed the degenerative changes which occur in the postganglionic fibers and their endings, Goecke & Beaufays (126) degeneration and regeneration in the nerves of transplanted ovaries, and Ford & Woodhall (116) certain clinical findings due to misdirection of regenerating fibers in cerebrospinal and autonomic nerves. Hollinshead & Finkelstein (177) removed the lower thoracic and upper lumbar sympathetic chains to denervate the adrenal medulla. Microscopic observations on the innervation of the medulla were made from month to month. As early as the fourth month, the plexus in the medulla was reformed in an apparently normal fashion. These fibers were formed mainly by regeneration of preganglionic and possibly some visceral afferent fibers from the lower thoracic and upper lumbar spinal nerves. It should be emphasized that the chains had been removed and any regeneration present took place by random chance and without any attempt to encourage it. This should sound a note of caution to all experimentalists involved in chronic experiments on completely sympathectomized animals. Under favorable conditions, *i.e.*, after crushing the splanchnic nerves in



the thorax or after they emerge below the diaphragm, Maes & Simeone (256) found functional evidence for regeneration of the fibers to the adrenal medulla 50 to 63 days after the original operation. As already mentioned, Simeone (329) studied the effect of regeneration of the nerve supply to the nictitating membrane on its response to epinephrine.

#### POSTERIOR ROOT EFFERENT IMPULSES

In the face of a rather formidable mass of evidence to the contrary in the earlier literature, there has been described a so-called "spinal parasympathetic system." This consists of fibers of intraspinal origin coursing via the dorsal roots to synapses around the dorsal root ganglion cells or out into the peripheral nerves. There are a number of variations of this concept which Hinsey (163) reviewed and presented experiments (165) which led him to doubt it. It is important to realize the significance of this problem as considered from a phylogenetical and embryological point of view. What is true for the frog may not be true for mammalian forms, for even somatic motor fibers may be found in frog's dorsal roots. Furthermore, if such a spinal parasympathetic system is present in mammals, it should have been described in studies of their development, but competent embryologists have failed to find such a system.

Fulton (119, p. 223) makes the statement, "That synapses occur in the posterior root ganglia is well established, but that they activate the posterior root vasodilators has not been proved." Reference to a paper by Barris (25), who made a thorough study of the atypical neurons in the dorsal root ganglia of the cat, will give the evidence that pericellular plexuses, surrounding dorsal root ganglion cells, are very rare in normal ganglia of cats. Their number was greatly increased in certain experimental and pathological conditions. Barris believed it probable that those seen in normal ganglia have a similar origin and represent proliferations of newly-formed fibers from the sensory ganglion cells themselves. This work of Barris is a quantitative approach to and a reiteration of the view held by Cajal (68). The evidence seems clear-cut enough that no functional significance can be attributed to these pericellular plexuses, either in the problem of referred pain or efferent dorsal root conduction.

In their counts of nerve cells and of fibers in the roots central

and distal to the fourth spinal ganglion of the bullfrog, Lucas & Miksicek (247) found about 3,500 cells which did not have central processes. In his analysis of the action potentials of the bullfrog celiac nerve, Bishop (36) determined that the "C" potential was about ten times greater than the one in the corresponding roots. The fibers, whose impulses gave rise to the large "C" potential, were shown to originate in the dorsal root ganglia, probably from cells without central processes. Degeneration experiments showed that stimulation of fibers arising in the dorsal root ganglia caused stomach and gut contraction but no vasoconstriction. He found no pathway by which these cells could be activated normally in the body. Bishop & O'Leary (37) have made a more thorough study of the pathways through the sympathetic nervous system of the bullfrog. They have conservatively withheld judgment as to the interpretation of these fibers from the dorsal root ganglion which cause gut contraction. Hasimoto (140) produced contractions of the toad's stomach by stimulation of dorsal roots.

While Barnes & Davenport (24) question the one to one ratio between fibers in the mammalian dorsal roots and the number of cells in the respective ganglia, Duncan & Keyser (101) have reported counts which give close approximations to this ratio in the cervical, lumbar, sacral and coccygeal levels of the cat. In the thoracic segments, there was a greater relative number of unmyelinated fibers in the dorsal roots and small discrepancies existed. An average of several ganglia showed 8,494 cells and 8,832 fibers. This four per cent excess of fibers over cells is small indeed and may fall within the error of the method.

Support for the spinal parasympathetic system has been continued in a series of papers by Kuré and his countrymen (196, 209, 210, 211, 212, 213, 214, 215, 216, 217, 276, 316, 317). Anatomical work which has been pursued on the dog, in which roots have been sectioned and degeneration allowed to progress, is open to the fallacy that there are aberrant ganglion cells scattered along the intradural and extradural portions proximal to the ganglia proper. Duncan & Crocker (100) have studied these cells in both the cat and dog in a quantitative manner. Unless complete serial sections of all the proximal stumps of sectioned dorsal roots are studied, any conclusions are open to question. Furthermore, even though the dorsal root ganglia are excised, the possibilities of regeneration into the proximal stumps are not removed (165). Fibers of the type

and numbers which Kuré has represented have no substantiation from reliable work in the older or more recent literature.

Barron & Matthews (26) contend that as many as 32 per cent of the myelinated fibers in the lumbosacral dorsal roots of the cat are collaterals of posterior funicular fibers which pass to the periphery through the spinal ganglion without cell stations. It is difficult to explain this error in interpretation but that it is fallacious can be said without reservation. Storey, Corbin & Hinsey (346) have performed additional experiments using the Marchi technique to search for degeneration in peripheral stumps and dorsal root ganglia at proper times after dorsal root section. Whatever may be said in criticism of the Marchi technique, its proper application should easily have shown fibers such as Barron & Matthews described and they were not present.

Young & Zuckerman (388) applied the experimental approach to this problem in monkeys. Their work did not substantiate Barron & Matthews. However, with an interval of fifteen to twenty-two days after section of the dorsal roots proximal to the ganglia, they found intact fibers, never more than four greater than three microns and small numbers of fibers less than three microns (from 0.85 to 2.94 per cent of the total number of myelinated fibers in the respective roots). They admitted the possibility that these fibers were regenerating ones (293). The possibility must be recognized that some fibers may be resistant to degeneration in spite of the fact that most of the others have undergone degenerative changes.

The significant papers bearing on the study of action potentials in relation to efferent components in dorsal roots are those of Matthews (266), Barron & Matthews (27, 28) and Toennies (355). Toennies has shown that after a sensory nerve like the saphenous is stimulated there are impulses which travel away from the spinal cord in the dorsal roots of the same and opposite sides. The impulses travel over large fibers as well as small ones and the potentials indicate that as many as 35 per cent of the alpha fibers in the root may be involved. The reduced reflex time for the homolateral reflex was 4+ msec., and for the contralateral one 1.5 msec. longer. Summation, facilitation and inhibition were demonstrable. Toennies states that, "The conclusion seems to be inescapable that fibers from the dorsal root ganglia make central synaptic connections with internuncial neurons of the cord in such a way

they they can be reflexly excited with a resulting discharge of impulses toward the periphery of the body." It could not be concluded from his evidence whether these impulses traveled over fibers which might also conduct sensory impulses to the central nervous system or whether they represent ones which conduct only in a centrifugal direction. The fact that large fibers are involved, that there is a relatively long central reflex time, and that there is no reliable evidence that such large fibers in the number required have an intraspinal origin, places their origin undeniably in the dorsal root ganglia. Thus, while this work shakes the Bell-Magen-die law and the concept of forward conduction in the nervous system, it still leaves the anatomical substrate for the make up of the dorsal roots untouched.

It must be said that no known function has been determined for for such efferent conduction. Pinkston, Partington & Rosenblueth (296) have suggested that such impulses may produce vasodilatation, an idea which has been advanced frequently in the older as well as the more recent literature. According to the evidence of Hinsey & Gasser (170), such fibers are ones whose potentials are found in the "C" spike and are therefore very small ones. However, future work under different experimental conditions may show that larger fibers are involved. Zuckerman & Ruch (391) and Hinsey (166) found that deafferentation of one hind limb in the monkey and cat caused the skin temperature to be lower in that limb than in the opposite normally innervated one. When the thoracolumbar pathways to both hindlimbs of the cat were interrupted, Corbin & Hinsey (86) could not determine any significant differences in the skin temperatures on the two sides. According to Hinsey & Phillips (172), deafferentation of one forelimb (cat) caused it to be slightly warmer than the opposite one with normal innervation. This certainly casts doubt upon any tonic vasodilator influence via the dorsal roots.

Wybauw (384) has summarized a study of this problem, which is too extensive to review properly here. His work is negative as far as a true reflex dorsal root dilator effect from the cord out in an efferent direction. Stimulation of dorsal roots and the resulting vasodilatation was accompanied by acetylcholine production as shown by its presence in the venous blood returning from the pads. Stimulation of dorsal roots is said to have produced a dilatation in the deeper tissues as well as in the skin. Axon-reflexes

mediated over branches of nerve fibers arising from cells in the dorsal root ganglia exerted an important control over the vascular bed of the cat's pads. The cells, whose fibers are involved in axon-reflexes at the periphery have central processes which are activated on dorsal root stimulation. These axon-reflexes are similar to those which have been described by Sir Thomas Lewis in the human skin.

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DEPARTMENT OF PHYSIOLOGY  
CORNELL UNIVERSITY MEDICAL SCHOOL  
NEW YORK CITY

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## THE SPECIAL SENSES\*

BY J. M. D. OLMSTED

*Division of Physiology, University of California  
Berkeley, California*

### THE EYE

*Eye movements.*—The study of eye movements has received new impetus from the introduction of standard photographic methods, particularly by psychologists, for the purpose of recording eye movements during reading. One of the more important physiological facts brought out in such studies is that the eyes make divergent movements during the fixation periods, and convergent movements between fixations, *i.e.*, the older assumption that both eyes make exactly parallel movements is not true (1). As one might expect, anoxemia interferes with ocular reading movements; at the equivalent of an elevation of 18,000 ft. there is an increase in the number of fixations, decrease in divergence, appearance of nystagmoid movements (2). Forced movements of the eyes follow certain brain lesions, *e.g.*, horizontal conjugate deviation is seen after section of the medial longitudinal fasciculus, the right posterior commissural nucleus, the right lateral nucleus of the globus pallidus and fiber tracts connecting the last two structures. Since there is no known direct path from the cortex to the pallidum, Muskens (3) concludes that there is no direct cortical innervation of eye muscles. Stimulation of the grey matter around the aqueduct of Sylvius below the cranial part of the anterior corpora quadrigemina gives either constriction or dilation of the pupil; loss of this area produces only transitory effects. If the central grey matter below the posterior commissure is stimulated, downward conjugate movements result; if the posterior part of the thalamus or medial part of the tectum of the anterior quadrigemina is stimulated, the eyes turn up. Loss of these areas produces permanent effects (4). Recovery of function after cutting and reuniting the oculomotor nerve in a chimpanzee proved to be imperfect. The movements were complicated by retraction of the formerly ptotic eyelid whenever there was an inward or attempted downward movement of the eyes. Attempted downward movement resulted in inward movement of the eye. These effects are to

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be explained as the result of undifferentiated, disorderly regeneration of axis cylinders into the distal stump of the nerve; since the oculomotor nerve serves not only four different extra-ocular muscles, but the levator palpebrae, the constrictor of the pupil and ciliary muscles as well, incoordination would result from an indiscriminate course of the new fiber endings (5). A study of the smooth muscle of the orbit of the eye shows that the arrangement is utterly different in the cat and dog from that in man. In the lower animals Müller's muscle can push the eye forward, in man it cannot by itself produce proptosis, while other smooth muscle behind the eyeball can (6).

*Cornea.*—That corneal ulcers follow injury to the trigeminal nerve has been known since the time of Magendie, but only now is it possible to give an explanation of this "trophic" effect. The newly accepted humoral theory of neural transmission postulates acetylcholine as an intermediating substance at certain nerve endings. The normal cornea contains a considerable amount of acetylcholine; when the trigeminal nerve is cut there is a marked lowering of the acetylcholine content, the degree of deficiency being parallel with the corneal damage, although the amount of unesterized choline is not diminished. Instillation of acetylcholine into eyes of patients suffering from keratitis neuroparalytica has had strikingly beneficial results (7). The cornea and vitreous humor are both found to have the normal serological properties of other vertebrate tissues (8). The living cornea is permeable in either direction to such salts as potassium iodide, sodium iodide, and sodium nitrite, although less so than if the epithelial cells are injured (9).

*Aqueous humor.*—The origin of the aqueous humor has been a matter of some controversy. Duke-Elder, by direct osmotic pressure measurements, and by the vapor-pressure method of A. V. Hill, had found in etherized cats that the molecular concentration of the aqueous humor was less than that of the blood by an amount which was compatible with the theory that the aqueous humor is formed by a process of dialysation. There were objections to this explanation of the origin of aqueous humor on several grounds, one being that patients with severe edema have normal intra-ocular pressure (10,11). Further experiments by Duke-Elder showed that the relations previously discovered were the results of the anesthetic; under amytal, or with a local anesthetic, the

aqueous humor has an osmotic pressure equal to 0.004 *M* NaCl greater than that of the corresponding serum, and the theory that aqueous humor is a simple dialysate is now abandoned (12,13). The aqueous humor of normal rabbits contains about 22 mg. per 100 cc. ascorbic acid (vitamin C), depending on the food content and the season of the year, less in autumn than in summer. Administration of glucose causes rapid increase, radiation rapid decrease of ascorbic acid in aqueous humor (14). Glucose, even in epinephrine hypoglycemia, is eliminated by way of the lacrimal glands. Elimination begins when the blood sugar is 30 to 40 per cent above normal, so that the threshold for those glands is lower than the renal threshold (15).

*Lens.*—The question of the relation of vitamins and hormones to the science of ophthalmology has been treated by Wachholder *et al.* (16), and their relation to cataract in particular by Judkin (17) and others (18, 19). Dietary deficiencies, even in trout (20), have been found to cause cataracts. The vitamin-C content of cataractous lenses is less than that of the normal lens, although there appears to be no correlation between the type of lens abnormality and the level of the vitamin-C content of the blood, or between age and the vitamin-C content of the lens, so that certain authors have been forced to conclude that low vitamin-C content in the lens may be the result rather than the cause of cataractous changes (21). The common method of producing cataract in the rabbit has been by use of naphthalene. In such eyes the ascorbic acid and glutathione content of aqueous humor and lens is decreased (22). The rat, which is now so widely used in dietary experimentation, readily develops cataract after feeding with galactose. A description of the histological changes occurring in such lenses will be found in an article by Sasaki (23). He concludes that the permeability of the galactose lens is increased over the normal, that there is no increase in the galactose content of the lens itself (although the sugar content of both blood and aqueous humor is increased) and that the production of lens opacities is due to the difference in osmotic pressure of the aqueous humor and the lens. Süllmann & Weekers (24) also believe that osmotic pressure disturbances play some part in galactose cataracts. Sasaki finds that, unlike the lens with naphthalene cataract, the lens with galactose cataract protects vitamin C from destruction by oxidation, since because of the increased permeability the reducing

agent, glutathione, easily leaves the lens. Bellows & Rosner (25) also find a loss of glutathione preceding the typical lens opacities caused by a galactose diet, but they believe that cysteine and vitamin C exert a retarding influence on the onset of galactose cataract. They find that not only galactose but dextrose diet as well will cause cataract in rats, but these sugars cause retention of glutathione by the capsule of the lens; therefore, they conclude, there is decreased permeability of the capsule (26). Disturbances in the content of vitamin B<sub>1</sub> have also been found in cataractous lenses (27). Here it is assumed that disturbances in metabolism of the glucosides are responsible for the condition, there being insufficient reduction of pyruvic acid to lactic acid because of the disappearance of vitamin B<sub>1</sub>. The relation of dinitrophenol to cataract has received much attention since in certain cases the recent use of this drug to reduce obesity has been claimed to result in the formation of cataract. When this drug was added to the diet of guinea-pigs (28) on vitamin-C deficient diet, and to that of rats (29) on a diet deficient in vitamins A and B<sub>2</sub> (G) no changes in the lens could be observed, although in many cases the experiments were continued until the animals were *in extremis*. Since the calcium content of the cataractous lens may be as much as 12.5 per cent higher than that of the normal lens, in spite of the calcium content of the bone and blood of the person with cataract being normal, it is of interest that the magnesium content of the cataractous lens is the same as that of the normal. It is possible, however, to increase the magnesium of the lens, particularly in the capsule and outer layers, of rats after long periods of feeding magnesium, but this seems to throw no light on cataract formation (30).

It is claimed that modern measurements show that the posterior face of the lens during accommodation changes both form (curvature) and position to even a greater degree than found by Helmholtz (31) and that the lens fibers in the surface and at the equator are not mere elastic fibers, but actually contract and relax like smooth muscle (32).

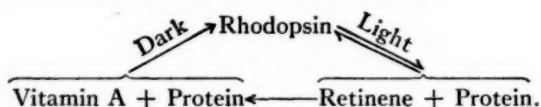
*Iris*.—Tissue culture experiments on embryonic iris tissue show that the sphincter muscle of the iris develops from a fold of the retinal layer, myofibrils appearing late (33). Isolated iris *in vitro* shows characteristic mydriasis or myosis upon the application of the appropriate drugs. Although the extent of movement is small

it is claimed that comparable data can be obtained by that method (34). One drug, phenylisopropylamine, produces marked dilation if applied to a rabbit's eye with the corresponding sympathetic nerve cut, and slight dilation of the other as well (35).

Studies on changes in pupil diameter by means of infrared photography have thrown some light on the reciprocal innervation of dilator and constrictor fibers. Dilation of the pupil of the normal eye during dark adaptation is the result of the passive pull of the iris tissue plus contraction of the dilator fibers. If the sympathetic is cut, dilation, which is only two-thirds maximal, is the resulting balance between the elastic pull of the iris tissue and "residual tone" of the sphincter. If both the sympathetic and the oculomotor nerve are cut, the pupil is fixed at slightly more than two-thirds maximal diameter by the pull of the elastic tissue alone. If the oculomotor nerve alone is cut, the pupil is fixed at its maximal diameter, and relaxation of the dilator fibers during light adaptation is too slight to affect the pupil (36, 37). In a somewhat speculative paper Cogan (38) suggests that the sympathetic system controlling the sphincter fibers tends to adapt the eye for distant objects, the parasympathetic controlling the radial fibers tends to adapt for near objects. This speculation leaves out of account the phenomenon of reciprocal innervation shown above. An attempt has been made by Haessler (39) to measure the changes in the pupil of the dark adapted eye during accommodation to near vision. This should, of course, involve separation of the effect of accommodation from that of convergence, but because of the method used this was not possible.

*Retina.*—The outstanding problems with regard to the retina center about the photochemistry of its pigments. Three photolabile retinal pigments are now known: (i) rhodopsin, the visual purple of frogs and mammals; (ii) porphyropsin, the visual purple of some fresh water fishes; (iii) iodopsin, a pigment of the cones (40). Rhodopsin always contains the carotenoids, retinene and vitamin A; the other systems involve other carotenoids. Rhodopsin appears to be a combination of retinene (visual yellow) and a protein of high molecular weight, above 200,000 as shown by its sedimentation constant which is the same for bleached as for unbleached visual purple (41). Light bleaches rhodopsin in a chain of reactions (40) the rate of which has been followed at different temperatures and pH values (42), the result being the separation

of the protein from the golden yellow lipid, retinene, which on further exposure to light yields "visual white", a colorless non-carotenoid substance (43, 44, 45). Vitamin A does not appear to be formed by the photo-chemical reactions of purified visual purple or of visual yellow, nor can it be detected spectroscopically in purified visual purple. It is present in the retina, chiefly in the non-bacillary layers (44). Extracts seem to regenerate visual purple only if they contain retinal pigment epithelium (45). The breakdown and resynthesis of visual purple can be directly correlated with the sensory phenomena of dark and light adaptation. They are schematically pictured by Wald (40, 46) thus:



The absorption spectrum of extracts of visual purple is of special interest because of its relation to the rod visibility curve for mammals including man (47). It has previously been assumed that the shift of the spectral sensitivity curve  $7m\mu$  towards the red was to be explained by the assumption that the refractive index of the solvents in the eye media was greater than those used for visual purple extracts. If the absorption is plotted as quanta the two curves coincide and there is no need of Kundt's rule (48). The classical absorption spectrum of visual purple has been reconsidered by Chase & Haig (49) who conclude that it is the absorption spectrum of the light sensitive portion only, while that of their own specially prepared unbleached purple solutions represent the molecule as a whole. Both scotopic (rod) and photopic (cone) visibility curves should take into account the recent accurate statement of the amount of physical energy reaching the retina (50). A comparison of the brightness of a light source acting directly on the retina with that of a light source of equal energy having first to pass through the eye media before reaching the retina shows that the latter is only 60 per cent of the former. Since the scotopic visibility curve is shifted almost  $50m\mu$  towards the short wave end of the spectrum, at low intensities the apparent brightness would be only about 50 per cent.

Studies of the relation of vitamin A to ocular disturbances are being pursued vigorously in both animals and man. Rats readily



show deficiency disturbances (51) even if adult (52). Vitamin A is found mainly in the dark adapted eye and hardly at all in the light adapted one. It is gradually lost during these adaptive changes and must be restored by the intake of appropriate food. Since it is necessary for the resynthesis of the visual purple of the rods, its deficiency is considered to be the cause of night-blindness. A description of the course of the development of the condition with diminished vitamin-A diet and the restoration of the sensitivity of the rods by administration of vitamin A or carotene is given by Wald *et al.* (53), who conclude that vitamin A is the precursor of cone visual pigments also. A mixed population, as in the city of Brussels (54), has been found to need at least 4000 international units of vitamin A per person per day in order to satisfy a standard test for dark adaptation, while in Washington, D. C. (55), 2100 to 3000 units sufficed. The discrepancy is probably to be accounted for by the difference in methods of making the dark adaptation tests. It might be expected that in vitamin-A deficiency there would be some abnormality in the pupillary response during dark adaptation, but none was noted (56).

Visual purple has long been known as the photosensitive pigment in the rods; the isolation of the hypothetical photochemical substance of the cones has presented greater difficulties. Both Chase (58, 59) and Studnitz (60) have obtained from the retina an extract which is evidently free from visual purple, but which is photosensitive, having maximum absorption in the yellow. This is claimed to contain the elusive cone pigment.

In fish and frogs, light, in addition to causing changes in visual purple, gives rise to retinomotor phenomena, pigment retraction, and elongation of the rods; reverse changes take place in the dark. The former action involves formation of phosphoric acid, the latter its disappearance. The effects are, however, not entirely the results of such purely chemical processes, but are controlled to a certain extent by nervous action (61). Stimulation of the anterior, posterior or intermediate lobe of the pituitary does not appear to influence the adaptation of the frog's retinal pigment to light (62).

The electrical response of the vertebrate eye to light (electroretinogram) according to Granit shows, after a brief latent period, first, a small a-wave indicating negativity of the posterior portion of the eyeball; then, a rapid positive b-wave; and finally a slow positive c-wave. Upon cessation of illumination, there is again a

latent period, then another positive rise, the d-wave. [The insect eye, *e.g.*, of the grasshopper, lacks the a-wave (63).] Granit (64, 65) considers that the electroretinogram is made up of three components, two of them positive, PI and PII, and the third component, PIII, negative. The positive component, PII, is associated with excitation, during which time the sensory epithelium is negative with regard to the ganglion layer; the negative component, PIII, is associated with inhibition, and the free ends of the sense cells are positive with regard to the ganglion layer. If during the electrical discharge following the removal of the light (the "off-effect") the retina is illuminated by a brief flash of light, there is a negative "notch," and studies of this phenomenon lead to the conclusion that some of the visual elements acting at "off" also respond to the flash, and are therefore incapable of responding in the second "off-effect" following the flash. This shows that elements which respond to re-illumination by inhibition are not the same as those put out of action by a preceding flash. This phenomenon of a special visual sense organ responding to a stimulus by inhibition is clearly shown in Hartline's (66) records of the impulses in the optic nerve of the pecten and in the responses of single nerve fibers of the optic nerve in cold-blooded vertebrates (67, 68). Cf. Sand (156, 157). In the pecten there are two layers of visual cells; fibers from the cells of the proximal layer give a discharge of impulses during illumination of the eye, fibers from the distal layer respond only to cessation or reduction of illumination ("off-effect"). Likewise in the optic nerve of cold-blooded vertebrates some fibers give only "off" responses. Here, however, the "off" response appears to have its site of origin in the ganglionic layers. Granit (69) has compared the electrical response of the frog's eye with the absorption curve for visual purple and finds that the curves fit well for longer wave-lengths, but for shorter ones the absorption of visual purple is greater than "physiological absorption."

Retinal changes in diabetics show that neither high blood sugar nor hypertension are in themselves the cause of retinitis diabetica (57).

*Vision.*—The relation of flicker to critical illumination and thresholds of vision has been studied extensively in the lower animals by Crozier (70, 71, 72, 73) and others (74, 75). The curves and resulting equations show among other findings that homologous results are obtained in man and those animals possessing both

rods and cones. Absence of rods is correlated with absence of certain portions of the curves. In man it is found that if electrodes are applied directly over the eyelid flickering phosphenes give a sensation of continuous bright light when the rate reaches 140 per sec. (76). Fusion frequency of the open eyes is, of course, influenced by the amount of illumination, and is a direct function of the logarithm of the proportion of the dark-interval to the total stimulus cycle. These results of Ross (74) are, he claims, impossible to reconcile with the photochemical theories of Hecht. Bartley (77, 78, 79) from his studies of flicker believes that the nervous system of mammals does not mirror exactly the photochemical activity of the sense cells; not only are adjustments made so as to admit perception of higher frequencies, but if the rate is reduced the flashes produce an impression greater than that expected from photochemical considerations (72).

The difference in the efficiency of light falling on different parts of the retina is readily shown by brightness threshold measurements. In parafoveal areas the efficiency varies with brightness, whereas in the fovea it is practically unaffected by it. The distribution of rate of change in efficiency in the parafoveal area is consistent with the hypothesis of pigment migration or mechanical movements of retinal elements (80), although it must be remarked that these phenomena have heretofore been associated with the eyes of cold-blooded vertebrates. The threshold for light in the parafoveal area decreases in an orderly fashion as the area of the visual field is increased (81). This signifies that at the threshold a constant number of elements respond and that this part of the retina is a relatively simple mosaic.

Visual acuity is defined as the power to see clearly or to discriminate detail in objects, and is usually measured by the angle subtended at the nodal point of the eye, or more practically by the angle subtended at the frontal surface of the eye, by the finest detail distinguishable. It is greatly modified by the intensity of the illumination of the test object, increasing slowly at lower intensities, faster at higher (82, 83). The best type of test object seems to be a broken circle, such as the letter C; a grating gives a maximal value about 30 per cent lower than for the letter C. There is a definite break at a visual acuity of 0.16, all values below this being mediated by the rods, those above by the cones. Detail perception appears to be a function of a distance rather than of an area (84).

By means of respiratory conditioned reflexes it is possible to determine the visibility curves for the rabbit. The curve for bright light is similar in shape to that for dim light, but the latter is shifted some  $30m\mu$  toward the red end, which shows that cone and rod vision in the rabbit closely parallel that of man (85). The optical condition of the atmosphere, the subject's space sense, his light sense and his power of dark adaptation all have an effect on acuity tests at low illumination. For night-flying the speed of one's dark adaptation is the most important factor, the amount is of next importance (86).

The accepted theory (Hecht's) that brightness discrimination is due to photochemical processes taking place in an eye adapted for a given intensity of light when it is exposed to a just discriminably higher intensity raises at once the question whether the Bunsen-Roscoe law holds here as well as for brief flashes of light. It is found to hold within the limits of a certain critical duration; at durations beyond this the Bunsen-Roscoe law is superseded by the relation:  $\Delta I = a \text{ constant}$  (87). The effect of adaptation<sup>1</sup> on differential brightness discrimination is shown in the finding that the differential threshold is lowest where the "test" object and "adapt" object are equally illuminated, but that the threshold is raised one hundred per cent by adaptation to illuminations greatly exceeding the "test" intensity (89).

A review of recent literature on dark adaptation has been made by Feldman (90) with special reference to the clinic. The following points should, however, be noted. The time for complete dark adaptation<sup>2</sup> is lessened as the difference between the intensity of light illuminating the "test" object and that of the bright light to which the eyes are exposed (91). Flashes of light during the course of dark adaptation cause only a momentary effect on the adaptation curve; there is rapid recovery to the level which would have been reached had there been no flash (92). Thresholds during the first twenty minutes of dark adaptation show summation for the two eyes for both peripheral and central vision. The binocular threshold is the same as the monocular threshold for a test patch of twice that area (93).

<sup>1</sup> A proposed theory of retinocerebral function with formulas for visual acuity and light and dark adaptation at the fovea (88) must be consulted in the original.

<sup>2</sup> In this connection the important paper of Hecht, S., Haig, C., and Chase, A., *J. Gen. Physiol.*, 20, 831 (1937) should be consulted.

Explanation of the phenomenon of after-images in such terms as "retinal unloading" does not, in the opinion of the author of this review, clarify our conception of the physiological processes involved (94, 95). Contrast, irradiation and simple after-images must in the first instance be dependent on the properties of the cones, the part played by the subcortical or cortical visual centers is obscure. The fact that the latency of negative after-effects is decreased with increase in the intensity of illumination of an adjacent retinal region is interpreted to mean that there is an inhibitory effect causing decrease in the excitation set up by the constant light. The locus of this inhibitory effect may be in the retinal synapses, the thalamus or the cortex (96). In general, Hyman's law that the inhibitory effect of a stimulus is directly proportional to its intensity does not seem to hold for the eye; there is relatively greater inhibition in bright light than in dim (97).

Perception of distance in monocular vision involves training, with diffusion circles playing a greater part than heretofore supposed. One can ignore the proprioceptive factor (98). Perception of depth in binocular vision is dependent on three factors: intensity, perspective and stereoscopy. Of these intensity is the weakest, perspective the strongest and stereoscopy nearly as strong as perspective. The latter two make the best combination of two factors (99). Perception of form in the case of geometric figures of small size viewed under different lighting is dependent on the number of sides of the figures and the size of the angles they subtend. The greater the number of sides and the greater the angles, the less readily is the form recognized. Increase in illumination gives better form perception than would be expected from the histology of the retina, hence Berger & Buchthal (100) conclude that not all the elements are acting simultaneously; some, which at lower illuminations would be in the relative refractory phase, at higher illuminations are able to act because the relatively refractory phase is now shortened. Both monocular and binocular egocentric localization, *i.e.*, the determination of the position of objects in relation to the observer, is, according to Bair (101), dependent upon four correlations involving the innervational states of the extra-ocular muscles and a psychological factor, the apparent size of objects.

The effect on vision of other stimuli such as sounds is to decrease the sensitivity of parafoveal vision (102).

Some recent definitions of terms designating the different types

of color-blind subjects may be useful: (i) Deuteranopes are those whose color system is reduced to yellow and blue, but who see color throughout the whole spectrum. (ii) Protanopes are those whose color system is reduced to yellow and blue, but whose spectrum is shortened at the red end. Their point of maximum brightness is shifted to the green. (iii) Anomalous trichromats are those who see Helmholtz' fundamental colors, but who are unequally sensitive to red and green. A recent comparison of different color-tests is given by Philip (103). Persons with normal color vision are found to vary from day to day with regard to the range of the yellow of the spectrum and the position of mid-yellow. There is a definite correlation between the "red"- "green" ratio and the intensity of "yellow" required to match the mixture; anomalous trichromatism may be detected by a departure from the normal limits (104). Determination of visual acuity and fusion frequency in the color-blind supports the contention that rods are the only light perceiving elements in the totally color-blind eye (105). It is claimed that in the protanope acoustic stimulation affects color sensitivity in the-blue region of the spectrum; in the normal eye it increases the sensitivity of the blue and green apparatus, lowering that of the red (106). An interesting account of a person blind from birth who acquired sight at the age of twenty-two is given by Colley (107). White was first appreciated; red was the first color differentiated, green next, the other colors following. The thoroughgoing mathematical treatment of the "intrinsic properties of the color domain" by Silberstein (108) will have greater appeal for the mathematical physicist than for the average physiologist.

#### THE EAR

At least two excellent reviews on the ear have appeared in 1938 (109, 110) so that it will be necessary to summarize here only the very recent work on this sense organ.

The histological modifications in the elastic fibers of the tympanic membrane at various ages have been studied by Zanzucchi (111). Between the external layer of skin and the internal mucous membrane lies the substantia propria which has an external layer of radial elastic fibers and an internal one of circular fibers. A third layer interlacing between these two has been described which is more evident in the posterior segment of the tympanum. In the foetus the tympanum is thick and vascular, the radial elastic fibers

being well marked, the circular ones less so. Up to about eight years the membrane is proportionately thinner and less vascular, the radial and circular fibers occurring in the same proportion; they contain collagen and their elastic nature is obvious. From nine to fifteen years these fibers stain like true elastic tissue, the radial fibers reach from the handle of the malleus to the tympanic ring, and the circular fibers are especially marked near the umbo. Until fifty there is little change. From fifty to seventy-five the substantia propria becomes still thinner and a deposition of sclerosing fibers occurs around the umbo and at the periphery; the elastic fibers are now less obvious and take the stain less well.

Even with total loss of the tympanic membrane it is claimed that cochlear potentials in man can be obtained if the sound is conveyed to the middle ear by special tubes (112). Circumscribed lesions in the tympanic membrane affect chiefly the ability to hear tones of from 256 to 4096 cycles per sec. both in man and in the cat (113). In the latter case, the test is made by means of cochlear potentials and the fact that the effect on these potentials in cats so closely parallels the loss of hearing in man is an excellent argument in favor of the theory that cochlear potential changes are a true index of changes in hearing acuity. Some authors, however, think that the reflexes of the stapedius are a surer index, since as yet we are not certain of the exact nature of cochlear potentials (114). Action potentials taken directly from the middle ear muscles themselves show that the reflex contraction of the stapedius takes place prior to that of the tensor tympani by 50 msec., but contraction of the latter overpowers and outlasts that of the former. For tones below 1000 cycles contraction of the two muscles lowers the efficiency of transmission; for tones close to 1100 cycles and for those above 2000 cycles transmission is unaffected; from 1300 to 1800 cycles transmission is enhanced the greatest improvement being at 1500. These findings lend support to the theory of the "protective" rather than of the "accommodative" action of these small muscles, *i.e.*, that they respond synergistically to all sounds to prevent excessive vibrations harming the inner ear, rather than that they "tune up" the transmission system (115). It is found that occlusion of the pharyngeal orifice of the auditory tube may lead to greater impairment in hearing high than low tones (116). A study of the effect of flight on the middle ear shows that as the atmospheric pressure is gradually reduced, a change of from 3 to 5



mm. Hg is required before any effect is perceptible. There is then a feeling of "fullness" and upon examination the tympanic membrane is found to be bulging. With a decrease of 15 mm. Hg there occurs an annoying click when the drum snaps back; the sensation of "fullness" now disappears. This effect is caused by the auditory tube being forced open by the excessive pressure in the middle ear. As the pressure is diminished further there is a succession of clicks until at relatively high altitudes, the air being so much less dense than at sea level, it gets through without difficulty. The auditory tubes therefore act like flutter-valves; on increased pressure they close and tend to stay closed (117).

The organ of Corti in man has been successfully measured by Hardy (118) who finds that the average length is 31.5 mm. (slightly longer for males than for females), the basal turn being 18.2, the middle 9.3, the apical 4.0 mm. respectively. The average number of turns is from  $2\frac{1}{2}$  to  $2\frac{3}{4}$ . The length increases slightly with age, but not the number of turns. The right turns are slightly longer than the left. That the organ of Corti is essentially a resonator is shown in Schulze's (119) experiments in which he thrust a small manometer of short natural period into the labyrinth at three different places in fresh postmortem preparation of rabbits' and human ears. A Helmholtz siren was used for test sounds of different pitch. For each position a distinct resonance curve was obtained, the frequency being higher the nearer the manometer was to the oval window. Resonating parts of the cochlea for high pitch were found to be more closely packed than for low. The highest intralabyrinthine pressure recorded was 4.5 mm. Hg. Pohlman (120) has objected to the present theories of the action of the cochlea. Since the cochlear liquid is incompressible and the otic capsule relatively inelastic, there must be a compensation area to "accept" the vibrations of the stapes. The prevailing theory is that longitudinal displacements in the scalae are translated into transverse vibrations in the basilar membranes. Pohlman claims that the mechanism of shock absorption in the internal ear has been mistaken for the displacements in the liquid essential for audition.

The question whether the cochlear response in the Wever-Bray phenomenon is the result of the electrical manifestations of physiological activity in that organ, or whether it arises not from the organ of Corti but from the polarization of membranes is still unsettled. Kupfer's (121) name is to be added to the list of adherents

to the latter theory. Hallpike (122) again states that a normal cochlear response can be obtained in the absence of hair cells and that a weak response or none at all may be obtained when these cells are morphologically normal. Guttman & Barrera (123) are to be added to the list of those who find, having carried on their experiment for as long as eighteen months, that after the cutting of the acoustic nerve and degeneration of the cochlear ganglion electrical responses are still obtainable.

Davis and his school (124) find that harmonics begin to appear at 40 to 50 db. above the threshold and increase in amplitude more rapidly than the fundamental as the intensity of the pure tone is increased. They find that even harmonics are affected by changes in the tensor tympani, but not odd harmonics. They conclude that aural harmonics arise from acoustical non-linearity and asymmetry of the middle ear. Wever & Bray (125) on the contrary hold that the graph showing the relationship of the logarithm of response to the logarithm of sound input is skewed at the top for both even and odd harmonics, and that the tensor tympani has no relation to the pattern of distortion, hence its site must be in the inner ear. Wever & Bray (126), again in opposition to Davis and his school, hold that there is a functional relationship between sound intensity and magnitude of cochlear response—it is a power function for all except the highest intensities where there is evidence of "overloading" of the ear, and here reflexes of the tensor tympani interfere.

The electrical responses of the auditory mechanisms of cold-blooded vertebrates have been neatly worked out by Adrian (127, 128). In the tortoise the Wever-Bray effect, *i.e.*, the general correspondence of electrical oscillations to sounds, is absent; in the alligator, where there is a membranous drum and true cochlea, the phenomenon is present. Adrian's results emphasize the necessity of a constant high temperature for the nervous mechanism of the ear, a condition which is realized in the mammal.

Quinine affects both parts of the acoustic nerve; there are degenerative changes in the ganglion spirale (of Corti) and in the vestibular ganglion as well, but the effects are more marked on the cochlear than on the vestibular system. Upon injection of this drug it has been observed, however, that there are spontaneous nystagmus and certain changes in reactions to rotation tests (129).

When one compares threshold for vibratory stimuli in a subject

over a period of time, it is found that the average range of fluctuation in the auditory field is about 15 db., and twice this for vibrotactile thresholds (130). When a note of subliminal intensity acts on one ear and the other ear is tested for change in threshold intensity, it is found that the total energy for the binaural threshold is the same as that required at one ear at the monaural threshold, *i.e.*, one ear supplies the deficiency of the other and the output from the two cochleas is summed centrally (131). Curves have been constructed showing the variations in thresholds of auditory stimuli necessary to awaken a sleeper at different periods of sleep. Following the onset of sleep there is an S-shaped rise in threshold for about twenty-five minutes; there is then maintained over a similar interval a period of relatively high threshold and finally rapid fall almost to the waking level in the next ten minutes (132). It is claimed by Fowler (133) that the assumptions underlying present methods of measuring acuity of hearing are not tenable. If hearing is deficient for faint sounds it is assumed that it is in like proportion deficient for loud sounds. This is true for purely obstructive lesions, not for "neural" deafness. Tests have been devised to show which type of deafness is present.

#### THE LABYRINTH

The effects of unilabyrinthectomy in the chimpanzee resemble closely the effects of this operation in man, *i.e.*, transitory symptoms including horizontal nystagmus, forced movements of the head, falling, all of which disappear later. Bilateral labyrinthectomy in monkeys at first influences the gait and they depend upon vision for compensation (134). The treatment of Menière's disease by intracranial division of the vestibular nerve appears to be justified by the results (135), and shows that the cause of this syndrome is the stimulating effect of degenerative changes in the peripheral cochlear and vestibular structures, rather than disorders in salt or fluid metabolism.

By use of the Horsley-Clarke stereotaxic instrument Buchanan (136) has found that cerebellar lesions interfere with neither the slow nor the quick component of post-rotatory nystagmus in guinea-pigs. If the animal is decerebrated at the level of the superior colliculus and the mamillary bodies there is likewise no disturbance of nystagmus, provided there is no hemorrhage, so that post-

rotatory nystagmus is possible with both the thalamus and the cerebral hemispheres gone. The effect of vision on post-rotatory nystagmus is shown in the shorter duration of nystagmus than if the subject is blindfolded (137). The whole question of eye movements in relation to the vestibular apparatus has been treated by Ohm (138), and he points out that slow rotation of a normal person in the dark does not produce nystagmus but only a tonic deviation of the eyes in the same direction as the current in the endolymph caused by this rotation. It is only on rapid rotation that nystagmus occurs. He summarizes as follows the ways in which stimulation of a single semicircular canal may cause nystagmus to the right; (i) ampullopetal lymph current in the right horizontal semicircular canal, (ii) ampullofugal current in the left, (iii) irrigation of the right external auditory meatus with hot water, (iv) of the left with cold, (v) destruction of the left labyrinth or left vestibular nerve. The cardinal point of his theory of nystagmus is that there is a main stimuli-emitting station located in the nucleus of the vestibular nerve, and he gives diagrams to show the possible neural pathways involved.

The question of whether otolith organs or the semicircular canals are involved in tonic reflexes is still in an unsatisfactory state; textbooks still refer to the "static" action of the former in contrast to the "dynamic" action of the latter. Löwenstein (139) shows that in the pike at least elimination of a single horizontal canal results in permanent asymmetry of the eye muscle apparatus, hence a tonic (static) function of the semicircular canals must be assumed. On the other hand Hasegawa (140) finds that in guinea-pigs both sacculi are necessary for reflex responses to vertical acceleration; here the otolith organs have a dynamic function.

Rotational changes are found to increase the amplitude and number of fast electrical oscillations led off from the cortex of the vermis of the cerebellum. Rotation also causes increase in fast oscillations and inhibition of slow waves of the electrocorticogram even if the cerebellum is extirpated (141). This is taken to mean that impulses from the labyrinth can reach the cerebral hemispheres by pathways not involving the cerebellum. Finally, it may be stated that the reaction time to vestibular stimuli in man averages 598 msec., 2 msec. slower if the rotation is to the left than to the right (142).

## THE CUTANEOUS SENSATIONS

Nafe in a series of papers (143, 144, 145, 146) has attacked the century-old (Müllerian) theory of "specific nerve energies," and advocates the "pattern" theory. His arguments are based partly on extension of the old observation that the central part of the cornea is sensitive to touch, pressure and pain, but not to heat, cold, or pain aroused by heat; and partly on experiments on the skin of the limbs involving Lewis' method of pressure block to shut off circulation. Heat and cold sensations on this basis are the result of vasodilation and constriction. Nafe's arguments have been refuted one by one by Jenkins (147), and in addition the latter cites many phenomena which are unexplainable on Nafe's theory. In his own studies on thermal sensitivity Jenkins (148) advances the hypothesis that thermal responses are the result of a "pseudo-reversible" reaction,  $A \rightleftharpoons B$ , with the interaction of a third substance C which is necessary for the recovery phase after stimulation. The length of the adaptation time in his scheme is determined by the richness of the supply of C. The variation in sensitivity of the skin on repeated testing has long been commented on (147), but that repeated stimuli on one spot on the skin by the usual von Frey pressure-hair should render a spot symmetrical to the test spot more sensitive and cause in it the disappearance of the lability of the threshold (149) is as yet unexplainable in physiological terms. Repeated stimulation of a pain spot leads to hyperalgesia, vasodilation and edema (150), the typical H-substance reaction of Lewis.

Zotterman (151) has thrown light on the question whether touch, pressure and pain are mediated by the same sense organs and nerves or by different ones. He finds that the type of action potential and the rate of conduction are different for a light stroke on the cat's paw, a heavy stroke and noxious stimuli, such as burning, pinching, etc.

The vibration sense of the skin of the rat is found to have an upper limit comparable with that of man, *viz.* 1800 to 2000 cycles (152). There is a question, however, whether the vibration sense is really a separate sense or merely a special manifestation of the pressure sense. Gilmer (153) has decided in favor of the latter since he finds that the vibration sense and the response to mechanical stimulation are inseparable. According to him the vibration sense is merely interrupted pressure sense.

The power of man to localize cutaneous pain or heat stimuli is greater if pain- or warm-ending stimulation is accompanied by tactile sensations as well (154).

The sensitivity of contact-endings in the skin of the frog is increased by exposure to light after injection with eosin, an example of photodynamic effect (155).

The discovery of the functions of the ampullae of Lorenzini in the dogfish by Sand (156, 157), *viz.*, that they are thermal sense organs, involves a principle which may prove of great import in the study of sense organs. These ampullae respond to cooling by acceleration of the rhythm of impulse discharge of their nerves, but to warming by inhibition of impulse discharge. This inhibitory action of certain sense organs on discharge of nerve impulses has also been seen in Hartline's (66, 67, 68) experiments on the eye.

#### SMELL AND TASTE

Elsberg (158) has perfected a method for quantitative estimations of the sense of smell. Application of his method shows that just before and during the menstrual period there is hyperacuity of the sense of smell; that there is summation in birhynal smell; that olfactory fatigue depends not only on the strength of the stimulus and its duration, but also on the condition of the cells *e.g.*, intracranial tumors increase the length of time of fatigue as well as raise the threshold. This method of measurement has been found useful in diagnosis of brain tumors (159). Elsberg's theory of olfaction involves the solution of odorous particles in the lipoid coating of the olfactory hairs. Both the force of impingement of these particles (the mechanical action of air pressure) and the molecular activities of the surface of the hairs (chemical changes) are involved.

The threshold of the sense of sourness for a number of mineral and organic acids is at a pH of 4.4. Addition of 0.2M NaCl does not affect the sour taste, but three per cent sugar or a concentration of saccharin of an equal degree of sweetness decreases the sourness of hydrochloric acid by fifteen per cent, and of a buffered hydrochloric acid solution by forty per cent. This is in spite of the fact that the pH has not been altered in either case. This effect is thought to have a psychological rather than a physiological basis (160). It is found that the more acid the saliva, the greater is the sourness of an acetic acid solution—a fact which cannot be explained

merely on the ground of a possible buffering action (161). The so-called "electric" taste, which involves a sour taste at the anode when the current is made and at the cathode when it is broken is explained on the assumption that the former is the result of electrolysis of the saliva; the latter direct stimulation of the nerve endings (162).

Certain clinical cases lead Schwartz & Weddell (163) to conclude that in some persons the greater superficial petrosal nerve is the pathway for taste for the anterior two-thirds of the tongue. This is the result of observations on one case where the sense of taste was lost when this nerve was cut, and on two cases where taste was preserved following section of the chorda tympani. The majority of cases, however, show that the classical pathway through the chorda tympani is the usual one.

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DIVISION OF PHYSIOLOGY  
UNIVERSITY OF CALIFORNIA  
BERKELEY, CALIFORNIA

## PHYSIOLOGICAL PSYCHOLOGY\*

BY E. R. HILGARD AND C. P. STONE

*Department of Psychology, Stanford University, California*

### PART I. THE CONDITIONED REFLEX<sup>1</sup>

BY ERNEST R. HILGARD

The importance which now attaches to conditioned reflex experiments and theories is a tribute to the energy and enthusiasm of two distinguished Russian scientists, Ivan Petrovitch Pavlov (1849-1936) and Vladimir Mikhailovitch Bekhterev (1857-1927). The literature on the conditioned reflex now totals some 1500 titles, estimated by Razran to be 43 per cent Russian, 30 per cent English, 13 per cent German, 11 per cent French, and 3 per cent in other languages. Razran's bibliography contains 1111 titles of which either the originals or abstracts are available in non-Russian languages. The titles are indexed under eight general headings, with a total of 115 sub-topics. This bibliography [Razran (4)] is an important aid for anyone undertaking investigations in special phases of conditioning. The classical sources of information about conditioned reflexes are the books by Pavlov (1, 2) and Bekhterev (1, 2). The details of the construction of Pavlov's sound-proof laboratory, and the specific procedures for preparing animals for experimentation, are given by Podkopaev. The present review emphasizes contributions appearing during 1937, with occasional references to earlier studies in order to give continuity. The more recent summaries in English are by Hull (1) and Razran (1, 2, 3).

#### THE PHENOMENA INCLUDED AS CONDITIONED REFLEXES

Some confusion results from extending the concept of the conditioned reflex to cover all manner of acquired behavioral modifications. Locomotor behavior in free situations in which the ani-

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<sup>1</sup> The annual output of studies in physiological psychology is of such proportions that only selected topics, and for these only selected references, can be reviewed within the allotted space. Somewhat greater space has been devoted to conditioned reflexes than to the other topics chosen because at the present time it is one of the three or four most active fields of investigation. Perhaps in subsequent volumes it may be feasible to devote additional space, in turn, to each of the more active fields.

mal's behavior is modified through the use of incentives, commonly called trial-and-error learning, is now often included as an illustration of conditioning. Youtz has introduced the expression 'Thorndikian response' to distinguish between the conditioning of a sequence of movements by rewarding them at the end and the more usual conditioning, which he calls the 'Pavlovian response.' Rewarding an instrumental series of acts follows more nearly the pattern of the law of effect (Thorndike; Carr *et al.*) than that of simple reinforcement. Youtz showed, however, that a simplified Thorndikian situation could be used to demonstrate Pavlovian principles such as extinction and spontaneous recovery. The situation used was that earlier introduced by Skinner, in which a rat depresses a lever in order to obtain a pellet of food. Conditioning is measured in terms of lever-pressing, although the unconditioned response is eating. The relationship of incentives to conditioned behavior which is instrumental in securing a reward, or in avoiding a noxious stimulus, has been studied by Brogden, Lipman & Culler. They found that locomotor conditioning in a situation in which running terminated the shock was more successful than in a situation in which running did not terminate the shock. Extinction was more rapid if, instead of merely omitting the shock, the shock was presented if the animal responded. Learning to avoid the shock by not responding is obviously a variety of conditioning not covered by the traditional conception that the conditioned reflex resembles the unconditioned reflex.

Several discussions have been addressed to the problem of including or excluding instrumental locomotor and manipulatory responses as examples of conditioned reflexes. One solution of the problem is to consider such acts as a second variety of conditioning, thus making of the Pavlovian and the Thorndikian situations two different orders of conditioned response [Konorski & Miller (1, 2); Skinner (1, 2)]. Other writers believe that there are objections to including the Thorndikian situation as a form of conditioning, since there are many aspects of it without analogies in more usual conditioned reflex experiments [Schlosberg; Tolman (1, 2)]. The possibility remains that the two situations are closely related in terms of their inner dynamics [Hull (2); Hilgard; Youtz]. This may justify the application of some of the principles discovered in the more usual conditioning experiment to other learning situations, but it does not justify calling all learning situ-

ations conditioned reflex experiments. It is evident that no sharp boundary can be drawn, and it is important that more work be done in transitional situations.

The desirable practice appears to be to reserve the name conditioned reflex for alterations of response in experiments following the prototype of Pavlov and Bekhterev. This defines the conditioned reflex experiment by its mechanical arrangements rather than by the physiological processes involved, since the latter may be similar in a variety of learning experiments.

In view of the experimental differences, it is rather remarkable how many of the general principles discovered by Pavlov in his work with salivary responses in dogs can be demonstrated in experiments with other animals, including man. Among the principles recently given specific quantitative testing in man are: generalization, including the effects of reinforcement, extinction, spontaneous recovery [Hovland (1, 2, 3, 4)]; delayed and trace reflexes, including the inhibition of delay [Rodnick (1, 2)]; the course of acquisition with reinforcement (Hilgard & Campbell; Kantrow); discrimination (Hilgard, Campbell & Sears; Hilgard & Humphreys). The experiments of Kantrow were done on infants; the others were performed on human adults. Menzies succeeded in conditioning a vasomotor response, recorded by temperature changes in one hand, caused by modifying the temperature of the other hand. The vasomotor change was conditioned to a variety of stimuli, including the sound of a bell and a buzzer, postural adjustments by the subject, words spoken by the experimenter and by the subject. In the end, some subjects could 'voluntarily' produce the vasomotor change by merely thinking of the visual substitute stimulus. The studies of Hovland and of Rodnick utilized the galvanic skin response, which, because of its latency characteristics and relatively involuntary nature, is appropriate for comparison with Pavlov's experiments. The fact that such responses are not introspectable or under direct voluntary control does not mean that they are free from the influence of verbally induced sets. On the contrary, symbolic reinforcement appears to be quite as satisfactory as true reinforcement in producing the galvanic skin response. That is, causing the subject to expect a shock is as effective as actually shocking him (Cook & Harris; Mowrer). In spite of these complications with verbal behavior in man, Pavlovian principles may be demonstrated very satisfactorily with this



response. Wickens demonstrated, in a study of conditioned finger responses developed by the use of electric shocks, an alteration of the response from extension to flexion when the position of the electrodes was reversed, without any reinforcement having occurred in the new position. The moving of the electrodes illustrates the importance in man of symbolic reinforcement, but this may also be demonstrated in dogs (Kellogg & Walker).

Attempts to do experiments after the analogy of conditioning using responses definitely at a higher level of organization have been reported for learning the names of pictures (Eaton), for paired associates learning (Peak & Deese), for turning a crank (Rexroad), and for lifting the finger at instructions [Hunter (2)]. Many similarities are reported, although there are also differences (Eaton).

The conditioning of sensory responses is reported by Bogoslovski, who combined a metronome with a light which produced a heightening of electrical sensitivity. After conditioning, the metronome produced a similar change in electrical sensitivity. Hallucinatory conditioning is reported also by Mowrer.

These experiments suffice to indicate the wide generality of conditioning phenomena. Failures to confirm Pavlov's principles have been reported less often than successes, but they are equally important in arriving at a satisfactory theory of conditioning. There have been several failures to obtain experimental extinction (Harlow; Marquis & Hilgard; Menzies). These bear on Pavlov's concept of inhibition, to be discussed later. Menzies found greater extinction for conditioned responses based on stimuli produced by postural adjustments than for responses based on non-postural stimuli.

#### THE ANATOMICAL LOCUS OF CONDITIONING

Pavlov considered his studies of conditioning to be investigations of the activity of the cerebral hemispheres. Although there were certain residual functions in dogs with complete cortical ablation, Pavlov denied that true conditioned reflexes could be formed in such animals. That certain of the crudely differentiated responses of the decorticate dog may be considered true conditioned reflexes has been demonstrated by Culler & Mettler and by Girden, Mettler, Finch & Culler. In their first decorticate dog they succeeded in establishing conditioned reflexes both to visual

and auditory stimuli; in their second, they established conditioned reflexes to auditory, tactile, and thermal stimuli. The reflex could be extinguished; an auditory discrimination between a bell and a pure tone could be established. The retention after removal of the striate cortex of visual conditioned reflexes formed in monkeys prior to the operation led Marquis & Hilgard to attribute these conditioned reflexes to subcortical mechanisms. The results were similar to those which they had found earlier in dogs. Visual conditioning in the absence of the striate cortex had been found in Pavlov's laboratory, but Pavlov attributed the results to a substitutive participation by other cortical areas, an interpretation which is no longer tenable. Satisfactory conditioning theory must hereafter take into account the complex interplay between cortical and subcortical centers.

A number of experiments have been performed in order to delimit the portions of the reflex arc essential for conditioning. An early experiment by Lang & Olmsted had suggested that the peripheral systems might be important in maintaining the integrity of the conditioned reflex. Provisional answers are now available to a number of the questions which may be raised.

1. *Can a conditioned reflex be formed if the unconditioned movement is produced by faradization of the motor nerve or of the motor cortex?* When the unconditioned movement is produced by motor nerve stimulation, conditioning has been unsuccessful (Hilgard & Allen), except when the movement produced is complicated by voluntary supplementation (Yacorzynski & Guthrie). An ingenious method of stimulating different portions of the central nervous system has been devised by Loucks. He imbeds a coil beneath the skin, with electrodes permanently in position to stimulate the desired area. Stimulation is by induced faradic current. The same points may be activated day after day without the risk of infection. With such a coil placed over the motor area, it is possible to produce 'unconditioned' leg withdrawals with regularity. Attempts to produce conditioned responses under such circumstances have led to somewhat ambiguous results, but it may be concluded that the method is essentially unsuccessful for conditioning [Loucks (1)]. Conditioning appears to take place readily only if there is some sort of neural input to the central mechanism mediating the response. Loucks (2) has also attempted to produce conditioning through the humoral stimulation of periph-

eral structures. He attempted to produce a conditioned hyperglycemia in rabbits through combining a buzzer signal with the injection of epinephrine. Assuming that epinephrine produces hyperglycemia by acting directly upon the liver through the blood stream, the experiment is a logical parallel to those producing responses by action on peripheral motor structures. Negative results led Loucks to confirm his earlier conclusion that "the crux of the conditioning process is the hooking up of the central excitations evoked by the conditional signal with the central neural impulses constituting the sensory pattern of the unconditional stimulus."

2. *Can a conditioned reflex be formed if the unconditioned reflex arc is interrupted peripherally, so that the response does not occur?* Harlow & Stagner found that under striate muscle paralysis induced by curare, striate muscle conditioning did not occur, although pupillary responses, active under the drug, were conditioned. They interpreted their results as showing that conditioning could occur only if the response took place. Girden & Culler repeated the curare experiment with some modifications. A slight twitch which could be elicited under curare could be conditioned, but its conditioning could not be demonstrated in the normal state; conversely, a conditioned response established in the normal state disappeared under curare. The results were explained as two varieties of conditioning, cortical and subcortical, usual conditioning being cortical, conditioning under curare being subcortical. The results are not out of agreement with Harlow & Stagner, in that responses could be conditioned only if they occurred at the time of attempted conditioning.

Light & Gantt, through crushing the motor nerve, prevented leg withdrawal during the conditioning of the flexion reflex of the dog. When the nerve recovered, the conditioned response appeared without further training. Other postural responses had taken place during conditioning while the limb was paralyzed, so that the results are not entirely clear. Upon recovery the leg movement might have been assimilated to the other postural avoidance responses. Settlege carried out conditioning procedures on animals in which sodium amytal anesthesia prevented movement and observed that conditioned responses appeared when the effects of the drug diminished. While no one of these experiments is strictly crucial, the probability appears great that conditioning does not

require the peripheral mechanism of the response to be intact at the time of conditioning.

3. *Can a conditioned reflex be formed without sense-organ stimulation by an unconditioned stimulus?* Successful conditioning has been produced by stimulating lumbar posterior roots, and the posterior spinal columns [Gantt (2); Loucks & Gantt]. It appears, however, that pain fibers must be involved if defensive conditioned reflexes are to result (Loucks & Gantt). Conditioned reflexes have also been formed by stimulating the lateral cerebellar lobe (Brogden & Gantt).

While these experiments show that the peripheral afferent system need not be intact for conditioning to occur, it remains possible that a conditioned reflex, established with normal afferent paths, might be destroyed if these were later interrupted. The Lang & Olmsted result has been denied, however, by several later experimenters (Raeva & Rappoport; Yuschchenko, Rolle & Pupko; Settlege & Harlow). Functional inactivation of the unconditioned stimulus through habituation does not necessarily destroy conditioning (Harlow).

It appears that the peripheral afferent mechanism is not essential to the integrity of conditioning.

4. *Can a conditioned reflex be formed without sense-organ stimulation by the conditioned stimulus?* Successful conditioning has been produced by substituting for conditioned sense-organ stimulation the stimulation of the spinal cord, the cerebellum, and cortical sensory areas (e.g., the striate area) [Gantt (2); Loucks (4)].

In summary, it appears that conditioning is essentially a central phenomenon, not always cortical, which may be characterized as a complex integration of afferent impulses eventuating in an organized motor discharge. For the integration to occur neither afferent nor efferent peripheral structures need be intact. Motor responses are so intimately related to the proprioceptive stimulation which they normally arouse, however, that learning in the presence of peripheral lesions may be expected to differ from that under normal conditions.

#### NEUROPHYSIOLOGICAL THEORIES OF CONDITIONING

Neurophysiological theories of conditioning attempt to explain the events within conditioning experiments on the basis of known or assumed principles of nervous action. Pavlov inferred

from gradients of response obtained from neighboring points on the skin that there was a spread or irradiation of excitation over the cortical area corresponding to the skin surface. Other senses were likewise supposed to have areal representation. The irradiation of excitation (or of inhibition) over the cortex was supposed to be followed by its reverse, a process of concentration. After a cortical process subsides it may be succeeded by the opposite process. This is known as induction. Conditioning, according to Pavlov, is to be explained in terms of the interaction of excitation and inhibition through irradiation, concentration, and induction.

The evidence for the wave-like spreading of inhibition and excitation from one region to another has been examined by Loucks (3) and found to be unsatisfactory. The concept of inhibition has been made a special point of attack [Wendt (1); Wenger].

Alternative speculations about the cerebral basis of conditioning, including physico-mathematical theories (Rashevsky; Houselholder & Amelotti), suffer from lack of confirmatory evidence about the action of cortical neurones. A few experiments have been directed at securing more detailed knowledge about physiological events going on in nerve and muscle groups. The inactivation of local cortical areas by repeated stimulation has been described as extinction (Dusser de Barenne & McCulloch); similar experiments yield electrical changes which offer two factors which may facilitate response, and two which may inhibit it (McCulloch). Beritoff believes that excitation is transmitted through specific neurone chains, while inhibition and facilitation are more general and proceed through the neuropile. He and his coworkers object to the Sherringtonian concept of the algebraic summation of excitation and inhibition. In some circumstances they may add, while in other cases they may interfere (Beritoff, Bakuradze & Narikashvili).

Drabovitch proposes a theory that conditioned reflexes are due to isochronism which is progressively set up between the cortical neurones and the subcortical and peripheral centers. He and his associates have published several studies on the relation between conditioning and chronaxy [Chauchard, Chauchard & Drabovitch; Drabovitch & Bahuault; Drabovitch & Weger (1)].

Several action potential studies of the responses made in conditioning experiments have appeared [Hilden; Prosser & Hunter; Hunter (1)]. To the extent that these deal with peripheral struc-

tures they contribute little to the fundamental problem, except to provide further testimony to the unlikeness of conditioned and unconditioned reflexes.

A phenomenon like extinction was demonstrated in the spinal rat. Tail responses to a tap on the tail decreased with repetition (Prosser & Hunter). The decrement was attributed to the internuncial neurones, in part because of the failure to secure extinction in the knee-jerk of the spinal cat in which an internuncial neurone is probably not involved. In more usual extinction of conditioned reflexes there is an increase in latency [Hunter (1)], but such an increase in latency was missing in the startle and spinal reflexes diminished by repetition. Therefore there is some doubt about identifying the two kinds of decrement.

Gantt (3) has studied the relationship between the amount of food ingested and the amount of saliva secreted as an unconditioned reflex. He finds a linear relationship,  $S = a + bQ$ , where  $Q$  is the amount of food, and  $S$  the amount of salivation. He believes the constant  $a$  to be a 'hangover' from the conditioned reflex. According to Gantt (4), the unconditioned reflex inhibits the conditioned reflex, rather than summing with it, as might be expected. Anochin & Straj, however, found that a conditioned motor response is possible while an unconditioned stimulus is acting. In their experiment the dog could be fed either from the right side or from the left. Conditioned stimuli were presented appropriate to each side. If the dog was eating food presented from the left side, a conditioned stimulus on the right could elicit a head movement in that direction. They therefore disagree with the interpretation that conditioned activity is inhibited during unconditioned activity.

Much remains to be done before a satisfactory neurophysiological theory of conditioning is possible. Many of the earlier speculations are invalidated because of their spurious assumption that the conditioned reflex is merely the old unconditioned reflex attached to a new stimulus. More detailed qualitative accounts of the events within conditioning have shown the responses to be so variable under different circumstances that many writers have preferred to discuss the behavior in terms of functional adaptation of the organism to its environment rather than in terms of neurophysiology. They believe that a neurophysiological account cannot be given until the behavioral acts are better systematized.

## FUNCTIONAL THEORIES OF CONDITIONING

Pavlov conceived of stimuli evoking conditioned reflexes as signals to the organism of agencies favorable or destructive to it. The fact that strict simultaneity of conditioned and unconditioned stimuli is unfavorable for conditioning and a forward order is most favorable supports the interpretation of the conditioned stimulus as a signal (Kappauf & Schlosberg; Hilgard & Biel). Signalization is a functional concept, not a neurophysiological one. Functional theories organize the facts of conditioning into understandable relationships; they do not substitute for neurophysiological theories, but supplement them by posing questions which neurophysiology eventually must answer. Among specimens of functional theorizing may be mentioned Wendt's interpretation of inhibition as a competition between reaction systems [Wendt (1)], supported not only by the observation of behavior during periods of so-called 'inhibition of delay,' but also by the long retention of conditioned reflexes when they are not subjected to competing learning [2½ year retention reported by Wendt (2)]. Wenger adds the notion of decrease in proprioceptive facilitation as an aid in explaining extinction. Guthrie interprets conditioning as a form of association by contiguity; Loucks (3) discusses it from the standpoint of psychobiology; Zener, viewing the total behavior in experiments like Pavlov's, offers a sign-urge explanation. Schlosberg distinguishes between conditioned reflexes which are essentially preparatory reactions and success learning; Culler gives a common-sense account of the service of conditioning to the organism, and includes a distinction between conditioning and other learning on the basis of the motivation involved.

Functional theories of conditioning lead easily to analogies to other forms of learning studied in psychological laboratories. Leadership in the attempt to systematize learning theory around principles discovered in conditioned reflex experiments has been given by Hull (2). Starting with basic definitions and postulates, theorems are proposed which may be tested experimentally. This pattern has been followed by Spence (1, 2) in accounting for discrimination. Applications have been made to memorization by Ward and by Hovland (5), to the maze by Zieve. Analogies based on conditioning have been objected to by Loucks (3) and by



Tolman (1, 2). Some of the problems are discussed by Kellogg. The different proposed relationships between conditioning and more conventional learning experiments have been summarized by Hilgard.

#### SOME APPLICATIONS OF THE CONDITIONED REFLEX METHOD TO OTHER PROBLEMS

*Sensory acuity.*—Culler and his associates have done an extensive series of experiments involving hearing in animals. The more recent reports are those of Ades, Mettler & Culler; Brogden & Culler; Culler, Willmann & Mettler. On the basis both of the cochlear electrical response and of hearing tests conducted by the conditioning technique, the resonance principle, proposing a spatial representation of pitch in the cochlea, is now validated.

The brightness visibility curve of the rabbit has been determined by Brown through conditioning respiratory responses. The curve is similar in shape to that earlier determined for dim visibility, but is shifted approximately 30  $m\mu$  toward the red end of the spectrum. This demonstrates color vision, which Brown believes to be indicative of the value of the conditioned reflex technique, since color vision in infra-human mammals has not been demonstrated unequivocally by other methods.

Olfactory and trigeminal conditioned reflexes have been studied in dogs by Allen. Oppenheimer & Spiegel have given a preliminary report on static and kinetic conditioned reflexes.

*Humoral conditioning and pharmacological reactions.*—There appears to have been little work on conditioned immunity since that summarized by Razran (2), Hull (1), and Metalnikov. Loucks (2) finds that responses to humoral agents can be conditioned only if the nervous system is involved in the 'unconditioned' reactions. The effects on the lever-pressing reaction of rats of caffeine, benzedrine, sodium bromide, epinephrine, ephedrine, insulin, phenobarbital, and some of these in combination, have been studied by Skinner & Heron and by Wentink. Drugs, particularly bromides, have been used in Pavlov's laboratory in therapy following experimental neuroses (Petrova). Extracts of adrenal cortex have been similarly used in the Cornell laboratory (Liddell, Anderson, Kotuka & Hartman).

*Experimental neuroses and psychotherapy.*—Some of Pavlov's animals appeared to be more subject to inhibition, others to

excitation. This led to his theory of types, reflecting the ancient classification of temperaments. The type theory has been questioned by Campbell, who finds a continuous distribution of ease of conditioning in man, and little interrelationship between ease of conditioning eyelid reactions and knee-jerks.

The clash of inhibition and excitation in the midst of a difficult discrimination, or in a period of delay, produced breakdowns in some dogs making them no longer suitable for experimentation. These states were interpreted as conditioned neuroses, and led to interpretations of human neuroses and psychoses on a similar basis [Pavlov (3)]. Sleep and hypnosis were interpreted as generalized states of internal inhibition (Pavlov & Petrova; Mishchenko). Experimental neuroses have been studied by Anderson & Liddell; Liddell, Sutherland, Parmenter, Curtis & Anderson; Drabovitch & Weger (2). Liddell and his associates have studied the neuroses of sheep and pigs. They find, in general, that signs of nervous tension appear when motor outlets are blocked. Drabovitch & Weger report neuroses in two dogs. Phenomena like those reported by Pavlov have been found in many laboratories [Gantt (1)], but the present status of the knowledge of type of nervous system in relation to experimental neurosis is very unsatisfactory, particularly because so little has been recorded of the earlier history of the animals used in the experiments.

Attempts to apply conditioning principles in psychoanalysis and psychiatry have suffered from an unwarranted use of analogy. Gesell, reviewing 57 references, finds the service of the conditioned reflex to the psychiatry of infancy very limited. Mowrer makes some suggestions regarding the relation between aspects of conditioning and aspects of repression and regression as studied by Freud. Schilder believes Pavlov's physiology too limited to cover the problems with which psychoanalysis deals.

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DEPARTMENT OF PSYCHOLOGY  
STANFORD UNIVERSITY, CALIFORNIA

## PART II. MISCELLANEOUS TOPICS

BY CALVIN P. STONE

### DEVELOPMENT OF BEHAVIOR

Interest in the development of behavior continues to embrace descriptive studies, which deal with sequence and schedule, as well as those which pertain to organization and inter-relationships. Youngstrom (1) demonstrated small but consistent differences in the developmental rates of 7 species of *Anura* and several species of *Amblystoma*. There were small temporal differences in the beginnings of sensory responses to light tactile stimuli in the different species; also, the earliest sensory responses were not correlated with exactly the same stages of motor development. Apparently genic factors are, in part, responsible for these differences.

Following conventional methods, Tuge (2) charted the development of behavior in pigeons. The first body zones responsive to external stimulation were the snout and upper neck, then came the shoulder, trunk, back, and hip in an antero-caudal progression; finally, the appendages were included. Spontaneous movements of myogenic origin first appeared between eighty-five and ninety hours of incubation; those of neurogenic origin, at ninety-five hours of incubation. First responses to tactile stimulation, consisting of flexion of the head, the neck, and sometimes the trunk, occurred at 123 hours. At about 133 hours there was participation of the tail and extremities with the trunk movements. At 145 hours the embryos began to display discrete reflexes of the tail, wings and hind limbs, without participation of trunk movements. Pecking action, mouth opening, rotation of the eyeballs, anal movements, and separation of the toes first occurred in conjunction with gross bodily movements; later on, they appeared as local reflexes. The author supports the well known conclusion of Coghill that partial behavior patterns develop out of the total behavior pattern and that only gradually is discreteness of response achieved.

Three intensive studies dealing with the development of special action systems (3, 4, 5) have appeared. Carmichael & Lehner (3) have charted the development of temperature sensitivity in foetal guinea pigs. At the youngest ages in which responses are found cold stimuli appear to be slightly more effective than warm stimuli.



From early to late stages there is little difference in the percentage of discrete, as opposed to generalized, responses to thermal stimuli. With advancing age, there are some shifts in the relative sensitiveness of the six areas studied, these shifts being in harmony with previous observations that the development of cutaneous sensitivity proceeds in a cephalocaudal direction and from proximal to distal parts of the limbs. The fact that some degree of thermal sensitivity can be demonstrated in the guinea pig during almost the entire foetal period implies that thermal regulation is of vital importance in this period of development.

Using motion pictures for recording, Ames (4) made a systematic study of the sequential patterning of prone progression (creeping) in twenty human infants. The following list of behavior items shows the sequence of development; the order of appearance of these items is quite inflexible:

(1) One knee and thigh forward beside the body; (2) Knee and thigh forward, inner side of foot contacting the floor; (3) Pivoting; (4) Inferior low creep position; (5) Low creep position; (6) Crawling; (7) High creep position; (8) Retrogression; (9) Rocking; (10) Creep-crawling; (11) Creeping on hands and knees; (12) Creeping, near step with one foot; (13) Creeping, step with one foot; (14) Quadrupedal progression, creeping on hands and feet.

Development of prone progression proceeds in a cephalocaudal direction and arm development antecedes foot development. Individual differences can be brought under three subclasses: (1) age at which a given stage is first manifested; (2) degree to which each stage is elaborated after it appears; and (3) tempo of individual movements and groups of movements. Practice does not hasten the progress through any one stage.

From the Yale laboratory came a volume by Gesell & Ilg (5) on the feeding behavior of infants. One of the most distinctive features of this volume is its portrayal of the development of feeding responses through seven age periods, ending with the second year of postnatal life. The authors are impressed with the evidence pointing to maturation, as opposed to learning, as the process by which the infant becomes ready to advance from stage to stage of feeding responses. Part three deals with the mental hygiene of the feeding period in a thoroughly common sense manner. There is very little inclination toward the Freudian hypotheses as to the interpretation of maladjustments arising from faulty training during this period. The book is a very significant psychological contribution to the literature of child development.

Of some theoretical as well as practical importance is the report of Melcher (6), who studied the development of 42 healthy infants that were prematurely born. They ranged in age from one month and a half to slightly over eighteen months. The Bühler-Hetzer tests were used to chart behavioral development. Their results indicate that up to five months of age these infants lagged behind the average for children born at term, but thereafter fell within average limits. A correlation of  $.41 \pm .08$  between birth weight and developmental quotient was found. Qualitative studies indicated that they were less retarded in sense perception than in postural control.

Typifying a growing interest in the organization of behavior is the classificatory and descriptive study of Pratt (7). He presents an excellent inventory of responses by human infants to stimulation at various stages of their development. With the aid of this inventory, he describes concomitant behavior, and indicates quantitatively the stimulus-receptor-effector relations of a given response to other responses at different ages of the infant. It affords him a quantitative medium for comparing one type of response with another. Pratt's study shows that the human infant usually manifests highly generalized, rather than highly specific, responses to external stimulation.

At the Institute for Child Welfare, University of California, two psychological approaches to the study of organization of behavior were made. Honzik & Jones (8) using correlational techniques with 127 boys and 125 girls, twenty-one months to seven years in age, found that mental and physical superiority were positively correlated to a small degree. Above average increments in mental ability, as tested, were positively associated with above average increments in height and weight. Bayley & Jones (9) suggest that environmental factors play a role of changing significance in the mental development of young children. They found with children of 59 families that prior to the age of eighteen months measures of environmental factors consisting of parents' education, family income, fathers' occupation, and other data entering into the sociocultural ratings are not significantly correlated with mental test ratings. Thereafter, the correlation coefficients are positive and increase in magnitude with age. For instance, the mothers' education correlated with the children's mental tests to the extent of .5 at the age of five years; fathers' income, with mental test to the extent of .3 at six years; and

total scores on the socio-economic scale, with mental test scores to the extent of .41 at six years. These findings show how early the intelligence stratification of a population becomes associated with socio-economic stratification in our present culture. Such a relationship at later ages has been known for many years.

#### BRAIN MECHANISMS

*Innate organization.*—In the female rat Stone (10) found apparently normal copulation, gestation, parturition, and maternal behavior (nesting, cleaning of young, retrieving, removal of young from a cold draft or from excessive heat, etc.) after they had been deprived of as much as 25 per cent of the cerebral cortex. Degrees of failure in one or more respects became increasingly great when lesions ranged from 25 per cent to 50 per cent. A more extensive and intensive study of this same kind was reported by Beach (11). He found that cortical destruction as low as even 10 per cent was accompanied by some evidence of lower efficiency in maternal behavior. Roughly, the aggregate of interference with maternal behavior was proportional to the extent of the lesion, with lesions varying from 1 per cent to 50 per cent of the total cerebral cortex. From these studies it is evident that the cerebral cortex participates in innately organized responses, although the precise manner is not known. In the main, Brooks (12) supports the earlier findings of Stone, to the effect that all of the neocortex may be removed in rabbits without suppressing the copulatory "instinct." If, in addition to the cortex, the olfactory bulbs were removed in males, failures to copulate were observed. Females, however, continued to mate and to ovulate after removal of all of the neocortex plus complete ablation of the olfactory bulbs.

*Higher mental functions.*—From over twenty excellent papers dealing with sense perception, learning, and higher mental processes as related to cortical functioning, two are selected for review as typifying important pioneer work. Krechevsky (13) found that normal rats can adopt an *Umweg* solution (in the visual mode) if that solution results in a more efficient performance in terms of distance to the goal. Small cortical lesions placed anywhere in the cerebral cortex greatly reduced the rat's capacity for *Umweg* solutions. So far, no correlation has been found between the size or the locus of the lesion and the amount of decrement, relationships that have been found repeatedly for maze and visual habits.

Working with what are called tests of reasoning ability in rats,

Maier (14) studied the effects of cortical lesions on "direction" in the solution of problems. Maier uses the term "direction" to designate the selective and integrative processes in the reorganization of experiences which antecede a solution whereby a goal may be achieved. To one group of rats he provided a training procedure which set up in them the necessary "direction" or organic set for the solution of a complex reasoning problem; to the other group this specific kind of training was denied. Both groups were operated and then subjected to post-operative training. The first group made scores that were well above chance, as a result of using the technique previously given them by special training; the second group was unable to acquire the required technique for solving the reasoning problem, and thus failed to score above chance. These data suggest that rats which in pre-operative training have acquired a technique ("direction") for the solution of complex problems may be able to utilize this technique postoperatively, although rats with similar lesions may not be able to acquire it *de novo* after operation.

#### BEHAVIOR RELATED TO NUTRITION

*Appetite.*—Using the self-selection method, Richter *et al.* (15) studied the ability of rats to compose a good diet for themselves by selecting the right kinds and amounts of purified foods. The following representatives of the important nutritional needs of the animals were presented: olive oil, sucrose, casein, cod-liver oil, wheat-germ oil, yeast, sodium chloride, calcium lactate, sodium phosphate, and potassium chloride. Choices by eight experimental animals, previously reared on a standard McCollum diet, suggest that rats have well-defined appetites for protein, carbohydrate, sodium, calcium, phosphorus, potassium, and the vitamins. Their selections were conducive to normal growth and reproduction. The average caloric intake was 18.7 per cent less than that of controls reared on the McCollum diet. An earlier study by the same authors (16) had shown that rats have a great craving for vitamin B<sub>1</sub>. When given access to an aqueous solution containing this vitamin, they consumed large quantities and strove for more. When its container was placed randomly among several others, they quickly found the right one. Presumably the vitamin was recognized by odor and taste.

Pregnant females (17), when allowed self-determination of the amount of sodium chloride ingested, consumed over twice as

much during the first ten days of pregnancy as during the preconception period, and over three times as much during the second ten days of pregnancy. Following parturition, the sodium chloride intake quickly dropped to the preconception level. In this connection, Barelare & Richter discuss the probable role of increased intake of sodium chloride in relation to decreased electrolyte content of the blood serum of pregnant females. This work suggests that there may be many other changes in the appetites of gestating females.

According to Richter & Eckert (18), parathyroidectomized rats had a strong preference for water containing 2.4 per cent of calcium lactate as opposed to ordinary tap water. After parathyroidectomy, their intake of calcium was 3.9 times greater than that of normal controls. Functional grafts of parathyroid tissue, established in the anterior chamber of the eye, caused the calcium intake to drop back to that of unoperated rats. Working with adrenalectomized rats, these same authors (19) obtained results by the self-selection method which indicate that the adrenals are concerned with the metabolism of all, or nearly all, of the electrolytes found in the normal blood serum. They state that "Adrenalectomy produced a definitely increased appetite (10-fold in some instances) for sodium lactate (3 animals) and sodium phosphate (4 animals) solutions, as well as for sodium chloride (13 animals)." Functional grafts of the adrenal cortex in the anterior chamber of the eye brought the intake back to the normal level. When a choice between tap water and chlorides of iron, magnesium, calcium, aluminum, potassium, or ammonium was required, the adrenalectomized animals showed no increase in appetite for the salts. In another experiment involving multiple choices, eleven rats on a low mineral diet were given an opportunity to choose sodium chloride, sodium lactate, sodium phosphate, potassium chloride, calcium lactate, or water. In this, "Adrenalectomy produced an increased intake of the sodium solutions, but also a small increase in potassium chloride. The mortality was reduced from 100% to 0." Again, rats maintained on the regular McCollum diet were given a wide assortment from which to choose: sodium chloride, sodium phosphate, sodium iodide, potassium chloride, ammonium sulphate, calcium lactate, and water. Adrenalectomy caused an increased intake of all except the sodium iodide. The relative proportions of intake of the different ions were about the same as the proportions normally found in blood serum. Following injections

of cortical extract (0.5 cc. "eschatin"), the mineral appetites of these animals returned approximately to their normal levels.

As a correction against the too ready assumption that appetitional factors alone determine the rat's choice of foodstuffs in a free situation, the illuminating experiments of Wilder (20) are cited. He found that 28 per cent of forty rats that had selected the short path of a choice maze having a long and a short path, and had been rewarded with a rachitic diet at the end of both paths, selected an antirachitic diet consistently when rewarded with this substance at the end of the long path. Their choice of long path persisted despite a water-pan obstruction and the introduction of blind alleys in the long path. The others, however (being a majority), did not give up the short path even though the antirachitic diet awaited them at the end of the long path. In a simple choice situation where an antirachitic and a rachitic diet were equally accessible, 68 per cent of a group of twenty-five animals selected the antirachitic diet in preference to the other. In the same kind of choice situation 75 per cent of a group of seventeen animals previously maintained on an antirachitic diet selected the rachitic diet consistently. In the same situation, 95 per cent of a group of twenty-two animals that had been maintained on a standard diet showed no consistent preference for either the rachitic or the non-rachitic diet. None of twenty-seven animals subjected to prolonged rachitic feeding for a period of twenty-six to thirty days prior to choice tests showed a preference for the antirachitic diet in the same choice situation. The foregoing results are disconcerting in the face of so many recent studies showing the seemingly automatic shifts from an inadequate to an adequate diet. They clearly show the importance of recognizing individual differences in appetites, and especially the influence of extra-nutritional factors associated with food-place habits, familiarity with a diet, and possibly still other non-appetitional factors associated with eating.

*Impairment of learning.*—It has long been assumed that severe general inanition impairs intelligence. Crucial experimental evidence that this is true is wanting. Recently Biel (21) reopened the problem by an extensive study on albino rats. He restricted the nursing time of the young and so limited the daily ration after weaning that at the end of ninety days their mean weight was only 35 gm. Maze learning, first with hunger and later with thirst motivation, was required while the young were between thirty-five and ninety days of age. No impairment of maze-learn-



ing ability was found. These results are considered especially significant because severe infantile inanition was imposed long before the cortical neurones had completed their growth.

Several investigators have claimed that qualitative deficiency results in impairment of learning ability. Stevens (22) working with a small number of rats, partially depleted of vitamin B<sub>1</sub> while nursing and trained on a maze later on, found a slight decrement in their learning as compared with that of controls. A fat-free, vitamin B<sub>1</sub>-free diet intensified the decrement. This led him to suggest that the fat-free factor, rather than the vitamin-free factor might be the vital one in impairing learning. The author also voiced a query as to whether the alleged impairment of intelligence in previous studies of this kind could have resulted merely from a disturbance in motor equilibrium resulting from injury to the vestibular nuclei, this disturbance giving rise to poor performances which were erroneously taken as evidence of defect in intelligence. (Stevens' study is particularly weak with respect to control of motivation; also the small number of animals renders the significance of differences doubtful.)

The relative effectiveness of early vitamin-B deficiency as opposed to later deficiency in impairing the ability of rats to learn a maze has been studied by Bernhardt & Herbert (23). The authors erroneously conclude that early deficiency produces greater impairment than later deficiency. Although they found small differences between the means of control and experimental groups, these were inconstant as to direction and not statistically significant.

Ziegler & Knudson (24) made a qualitative analysis of activity in rats after they had recovered from rickets. Group I came from mothers that had been fed a rachitic diet before, during, and after pregnancy. Group II had rickets between the ages of forty and seventy days. Group III consisted of normal controls. All of the animals were trained on a Carr maze that had been used previously by Maurer & Tsai in their pioneer studies on the effects of vitamin-B deficiency. From the meager data provided by their small groups the authors concluded that the animals afflicted by rickets early in life were impaired more than those afflicted later in life. So inconsistent were differences between controls and experimental animals, however, that this conclusion must be regarded as extremely tentative. It is regrettable that in what appears to be an important problem the number of animals was



too few to separate clearly the influence of dietary factors from adventitious factors which give rise to individual differences in normal animals.

#### STUDIES BEARING ON DRIVE AND MOTIVATION

*Drive.*—The most unique technical studies of animal drives appearing during the year are those of Anderson on the interrelationships of drives. Using 22 different tests designed to measure three fundamental drives (sex, hunger, thirst), Anderson (25) applied them to 50 adult male rats. Only 67 out of a total of 190 correlations between different measures were statistically significant, and the majority of these were quite low. Measures from a one-hour copulation test correlated with those from a test of hunger drive to the extent of .39; with those from a modified Columbia obstruction test of sex motivation, to the extent of .51; with various activity measures, including the activity drum, to the extent of from .24 to .34; and with time and error scores from a 12-unit multiple *T* maze, to the extent of .66 and .77 respectively. The latter were among the highest correlations obtained, although, on *a priori* grounds, high correlations between these scores were least expected. In a second experiment (26) involving 47 measures of drive and of learning, the correlations were somewhat lower in general than those of the first study, but in the main there was essential agreement. Anderson's data make one hesitate in describing an animal as relatively weak or as relatively strong with respect to any specific drive (thirst, hunger, sex, etc.) because the animal's rating depends so much on the measures used and the conditions under which they were applied.

Proceeding along lines developed in his earlier work, Bruce (27) made a study of maze performances by rats in which the acuteness of hunger or thirst was reduced by allowing them to partake of food or water for ten, twenty, forty, or eighty seconds prior to starting the trial. This anticipatory reward led to better performances than those obtained when preliminary sampling of the incentive was not allowed. The improvement was slightly greater with thirst than with hunger. Bruce also found that if the sample was incongruent with the paramount need of the animal there was no beneficial effect on performance, such as that observed when the sample was congruent. The theoretical implications of these results for goal-gradient hypotheses (28) and for conceptions of the laws of learning are indicated.

*Energy output.*—Ingle (29) found that rats lose their drive for enforced work within a few hours after the anterior lobe of the hypophysis is removed. The relation of this decrease in energy output to suprarenal functioning is indicated by the fact that injections of cortin quite perceptibly reduced the work decrement associated with hypophysectomy, although the performance is never restored to the level of normal animals. Gordon *et al.* (30) found that over half of a group of 32 patients suffering from neuromuscular asthenia showed clinical improvement following treatment with cortin, in the sense that they were much less subject to muscular fatigue than before treatment.

*Sex behavior.*—Precocious activation of masculine behavior has been found by Morito (31) and Hamilton (32) in baby chicks that received daily injections of testosterone propionate. Hamilton's chicks began to crow and to show evidences of pugnacity at the age of ten days. They were slightly retarded in their physical growth as compared with normal chicks. Stone (33) injected young rats beginning with the age of twenty to twenty-five days. Some of them copulated in characteristic form at the age of twenty-eight days, this being from six to seven days earlier than that reported for the more precocious in a group of normal rats. Not all of the young males responded to the injections of the male hormone by precocious display of sexual aggression. For this no satisfactory explanation can be given, as yet.

Shapiro (34) observed the effects of testosterone propionate on the sex development of young male rats castrated prepuberally. Five males (30 to 50 gm.) were castrated before their sexual organs showed the characteristic pubescent changes. Then they were isolated for nine weeks before being tested for sexual activity. Eight days after beginning daily injections of 0.5 mg. of testosterone propionate there were unmistakable evidences of copulation. Stone (35), working with adult rats that had been castrated for approximately eight months, found that two or three injections of 0.125 mg. of testosterone propionate restored copulatory activity within three or four days; ejaculations did not appear, however, until eight or nine days after the first injection. Withdrawal of the hormone was followed by a measurable decrease in sexual activity within a week or less, but with a few injections of the hormone there was a return of sexual activity. Related to these results are those of Stone on impotent male rats (36) and those of Hamilton (37) for a 27 year old medical student whose body configuration

and sexual organs resembled those of a prepuberal castrate. He had never experienced ejaculations and only infrequent erections prior to treatment with the synthetic male hormone (testosterone acetate and propionate). Following treatment, his physical and mental virility were so enhanced that he was capable of sexual intercourse. This ability lapsed soon after cessation of daily injections. Control injections of oil failed to cause effects resembling those produced by the hormone. Also, other controls of suggestion seemed to have been adequately handled. Cases of psychic impotence also seemed to be benefited by injections of testosterone propionate (38).

Ball (39) induced masculine behavior in adult female rats by injections of testosterone propionate. The behavior described was not quite as complete in details as the pattern of males, but presumably some minor detail of the injections is responsible for that. In the case of males castrated sufficiently long for waning of sexual activity, Ball (40) revived their potency to a high degree by injections of female hormone, Progynon B (Schering). Only two of the six castrates, however, exhibited the ejaculatory reflex. Stone's work with castrated males (35) suggests that testosterone propionate is more effective than female hormone in reactivating castrated males.

Reports confirming the induction of broodiness in fowls and maternal behavior in rats by injections of prolactin have come from Riddle's laboratory (41). The report of Leblond (42), however, indicates that further work with the rat must be done before responses that are really maternal in character can be separated from those that presumably are incited by social factors or heat regulatory mechanisms. His results indicate that virgin rats quickly form the habit of retrieving infant rats under certain experimental conditions.

#### FACTORS RELATED TO MENTAL WORK AND EFFICIENCY

*Anoxemia.*—Recently much fundamental work has been done on the relation of oxygen deprivation to efficiency of mental work. Kraines (43) found a definite drop in intelligence score of subjects breathing 10 per cent oxygen (corresponding to an altitude of 20,000 feet). Subjective opinions of their abilities were unreliable under these conditions. Apparently, individuals classed as asthenics showed a greater drop in intelligence scores than those classed as pyknics (statistical evidence supporting this obser-

vation not given). McFarland (44) gave a battery of mental and motor tests to ten men during a period of three months while they were at various altitudes up to 20,140 feet in the Andes. There were perceptible differences in the means of performance at 15,440 feet; these become more marked at 17,000 feet or more. Although significant changes in psychological and physiological processes were found, there was no significant correlation between the measures of mental and physiological processes in this small group of individuals. The investigators ascribe the decrement in psychomotor efficiency to chemically induced cellular changes in the central nervous system due to lack of oxygen in the blood. An experimental study of reading (45) by adults subjected to oxygen concentrations corresponding to 15,000 and 18,000 feet brought out several types of incapacities. There were decreases in the speed and comprehension of materials read, a significant increase in the number of fixations and regressions, and an accentuation of abnormalities of adjustment of the eyes. Although at the end of one hour at 12.5 per cent oxygen concentration (corresponding to 13,000 feet elevation) there was some evidence of acclimatization, there was no evidence of acclimatization at concentrations corresponding to 18,000 feet. Photographic records of eye movements are very sensitive indicators of the first deleterious effects of mild anoxemia.

In a general way, the decrements in mental work found by Gellhorn (46) in subjects suffering various degrees of anoxemia are in agreement with those just cited. When there was a decrement in mental work, there was also evidence of loss in motor efficiency for acts depending upon cortical functions. If, however, the same low concentrations of oxygen were inhaled in the presence of 3 per cent carbon dioxide, the disturbances of association, of memory, etc., completely disappeared (47). Similarly, under this condition there were no decrements in muscular coordination, discrimination of visual intensity, and other physiological functions. The second part of Gellhorn's study (46) is an analysis of the effect carbon dioxide has on anoxemia. He concludes that the beneficial effects are based on:

its stimulatory effect on respiration whereby the alveolar oxygen pressure is raised; the potentiating effect of carbon dioxide and oxygen deficiency on the vasomotor center; the potentiating effect of carbon dioxide and oxygen deficiency on temperature regulation.

*Drugs.*—The relation of benzedrine sulphate to mental ef-

efficiency continues to attract the attention of experimenters, although for the most part the experiments have not been comprehensive or crucial in character. There seems to be general agreement that the subjective feelings of well-being are enhanced, but no general agreement as to the improvement of mental efficiency. Molitch & Eccles (48) tested 93 boys between the ages of eleven and seventeen years. Thereafter, before undergoing retests some were given control injections and others were given a dose of benzedrine sulphate. Those receiving the benzedrine sulphate improved the most. (Evidence that the obtained differences are significant is not provided.) Using multiplication problems as a measure of mental efficiency, McNamara *et al.* (49) tested the influence of benzedrine sulphate on the mental efficiency of six undergraduate and four graduate students. They found that on the average there was no significant increase or decrease in the number of problems solved or the errors made when pills containing 20 mg. of benzedrine sulphate were pitted against control pills of 20 mg. of lactose. The number of subjects in this study is small, yet the controls exercised were good; hence it seems probable that the influence of the drug on mental efficiency is correctly described. Wilbur *et al.* (50) administered benzedrine sulphate orally to 100 patients in whom there was the diagnosis of chronic exhaustion, depression, or psychoneurosis. The immediate effects were considered beneficial in 80 per cent, 70 per cent, and 46 per cent respectively, and in certain instances they were spectacular. Continued administration brought less favorable results; 50 per cent of those having chronic exhaustion and 25 per cent of those who were depressed continued to show benefit from one to eight months. No improvement in the psychotic disorder seemed to be noticeable.

The effects of sodium phenobarbital on the learning ability of rats were studied by Williams & O'Brien (51). A total of 55 animals was divided into equated groups and trained on a 10-unit U maze. The animals were made thirsty and allowed water as a reward at the end of the runs. The dose of the drug was 0.087 gram per kilo of body weight. In terms of time and error scores, the treated animals were significantly poorer than the controls. Re-learning after an interval of six weeks brought out an inferiority of the group receiving the drug that was less pronounced but in the same direction as that found in original learning.

Two studies of the effects of tobacco smoke on learning in rats

are deserving of notice. Phillips (52) found no decrement in learning by rats subjected to tobacco smoke. Pechstein & Reynolds (53), however, found that rats exposed to a limited amount of smoke excelled all normal groups in learning, but with excessive quantities became inferior to normal groups. The detrimental effects were increasingly apparent in successive generations, leading to incapacity for learning by the fourth generation. They also observed that a limited amount of smoke made rats more prolific than normals, but that prolonged exposure of these same rats to smoke resulted in reduction of size of litters and viability of offspring. Stunting effects were increasingly transmitted to the progeny of successive generations. This study, remarkable for its positive claims of influence of tobacco smoke, is lacking in some of the crucial controls one needs to assess it accurately. It would be well worth careful repetition.

*Bodily tension.*—Following Bills' 1927 study (54) of the influence of muscular tension on mental work, there has been increasing interest in this fundamental subject. Bills & Stauffacher (55) reported that experimentally induced muscular tension speeded up the rate of solving arithmetic problems, although it had no significant effect on errors. They further concluded that tension was more beneficial to poor performers than to good performers. Their study does not clearly demonstrate, however, that the beneficial effects are due to tension, *per se*, rather than to an added incentive for the person to complete his disagreeable task. In this same study they reported that no beneficial effects were found when the subjects were required to solve detectograms, a type of problem that was highly novel to the subjects. To this finding with detectograms, the implication of Freeman is *apropos*. He implies (56) that a single degree of tension such as that found optimal for the solution of arithmetical problems may not be optimal for a different type of mental activity. Presumably, experimentally induced tension facilitates mental work under certain conditions and inhibits it under others. This fact makes it extremely hard to generalize from one situation to another. Freeman (57) lists the following as factors which should be taken into account in studies of the effects of induced tension on mental performances: practice, age of subjects, major locus of the tension in relation to the reaction-pattern, and different temporal relations of the induced tension to the performance of the task.

Stauffacher (58) had subjects memorize nonsense syllables while pulling on two handles attached to ropes that supported various weights in an attempt to relate the level of mental work to the amount of experimentally induced tension. When the tension equaled one half the exertion of the subject at the end of sixty seconds of pulling, he obtained a significant improvement in memorization rate. Tensions amounting to one-quarter and three-quarters of the base led to a small but not significant increase in memorization rate. Stauffacher infers that extremely high and extremely low degrees of tension may result in a decrement in memorization. (This assumption was not verified experimentally.) Also, he stated that poor subjects improved more under tension than good subjects. (This statement is based merely on small differences in the means, without consideration of the unreliability of the differences.) As a third part of his experiment, Stauffacher tested the influence of tension on savings scores when some of the materials learned were relearned after an interval sufficient for forgetting. Neither clearly beneficial nor detrimental effects for the groups as a whole were demonstrated, although again there was the suggestion that good learners are adversely affected and that poor learners are benefited during the relearning phases of the study.

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DEPARTMENT OF PSYCHOLOGY  
STANFORD UNIVERSITY  
CALIFORNIA

## KIDNEY\*

BY HOMER W. SMITH

*Department of Physiology, New York University College  
of Medicine, New York City*

### ANATOMICAL STUDIES

*Macula Densa and Polkissen.*—No review of the physiology of the kidney can avoid a brief reference to recent anatomical observations on the *macula densa* and *Polkissen*, and their relation to the glomerular apparatus. The *macula densa*, first described by Zimmerman (206), consists of a specialized region of the distal (?) tubule, where the otherwise cuboidal tubular cells acquire a columnar, palisadic structure, and form a plaque in direct contact (*i.e.*, with no intervening capillaries or connective tissue) with the "juxtaglomerular apparatus" of the afferent arteriole. Just before this vessel enters the glomerulus it undergoes a marked change in structure. The internal elastic membrane disappears, the endothelium becomes discontinuous, and the muscle cells are overlaid with or partly replaced by a cushion of myo-epitheloid cells which form a continuous cuff about the arteriole. This myo-epitheloid structure was first described by Ruyter (145a) in 1925, named *Polkissen* by Zimmerman (206) in 1933, and is designated by Goormaghtigh (72, 1937) *l'appareil neuro-myo-arteriel juxta-glomerulaire*. Goormaghtigh includes in this term similar myo-epitheloid cells on the efferent arteriole, which, however, are always in isolated groups; and, in addition, a compact group of non-myoid, perhaps neural or secretory cells located in the angle between the afferent and efferent arterioles. Numerous nerve fibers are present in this apparatus. This "juxta-glomerular apparatus" and the adjacent *macula densa* constitute a device by which the tone of the afferent and perhaps the efferent arteriole can be regulated either in relation to the local arteriolar pressure or to changes in the volume, pressure, or composition of the urine, acting through the *macula densa*. The juxta-glomerular apparatus has been shown

\* Received December 1, 1938. An effort has been made to include in the following chapter all physiological papers published since the writer closed the bibliography of his monograph (167) in the Spring of 1937. In most instances the October or November journals of 1938 have been available.

to be present in all the common mammals and in man, and assumes increased interest in view of the evidence that some mechanism exists within the kidney which tends to regulate the renal blood flow independently of the arterial pressure. (See section on renal blood flow.) [For bibliography see Goormaghtigh (72a, b), Becher (8) and Elaut (41).]

*Athrocytosis, etc.*—The phenomena of athrocytosis has been reviewed by Gérard (62). This is the property of the proximal tubule to absorb electro-negative colloids (trypan blue, carmine, hemoglobin, etc.) through the apical surface of its cells. There is no evidence that substances thus absorbed are returned to the blood, though Gérard considers the phenomenon to be a functional vestige of a once active reabsorptive process. Athrocytosis may, however, play an important part in the degenerative changes in the tubules which accompany lipid nephrosis, albuminuria, hemoglobinuria, etc. (See section on proteins.) One interesting point not hitherto suggested, so far as we are aware, is that the absorption of colloids by the proximal tubule suggests that the essential locus of the "impermeability" of the tubule cells to small molecules may be at the basal rather than the apical membrane. It is amply demonstrated by the process of athrocytosis that the mere presence of dyes, proteins, etc. in the tubule cells affords no information upon the processes of tubular reabsorption and excretion.

Kempton (93) has shown that when the arterial circulation is completely occluded in the frog (Nussbaum's experiment) no urine is formed after the injection of urea. Previous experimenters have overlooked collateral arteries from the interior mesentery or iliac artery via the ureters, which vessels are dilated by urea. It is apparently impossible to obtain purely tubular urine by Nussbaum's experiment.

The possibility of an extra-glomerular blood supply to the renal tubules is reopened by Spanner's (173) description of arterio-venous anastomoses in the normal human kidney. Fuchs & Popper (56, 57, 58) discuss the circulation of renal interstitial fluid and offer a speculative explanation of water diuresis in terms of hydrostatic-oncotic pressure relations. Other anatomical papers must be cited only by subject: statistical data on the weight of the normal human kidney (190), of the cat kidney (78) and on the glomeruli of mammals (146); the secretory area of the human glomerulus (15); glomerular count in human kidney (124); the renal tubules

of *Necturus* (94); the peritoneal funnels of the frog (144); cyst formation in fish kidneys (76); cytology of the proximal tubule in the cat and dog (54); vascular supply of human kidney (143); and development of collateral renal circulation in dog (205).

#### GLOMERULAR FILTRATION

The physical factors (arterial, venous and urinary pressures, etc.) involved in the formation of urine have been discussed in an important review by Winton (204).

*Inulin*.—When inulin is perfused through the renal-portal vessels of the frog kidney it does not gain access to the lumen unless the tubule has been injured. When the kidneys of dogs and rabbits are perfused at pressures too low to effect filtration (25 to 30 mm. Hg) with blood containing inulin, phenol red, diodrast and hippuran, and then subsequently perfused at higher pressures with blood containing none of these substances, the urine formed in the second period contains phenol red, diodrast and hippuran, but only traces of inulin. It is concluded that inulin is not excreted by the tubules in these animals (141).

No substance other than inulin is known as yet which fulfils the physiological requirements for measuring the rate of glomerular filtration in man; but the fact that at elevated levels of vitamin C, which is filtered and reabsorbed by the tubules, the vitamin C-inulin clearance ratio rises to 0.94 (140), coupled with the previously established fact that at elevated plasma levels of creatinine, which is in part excreted by the tubules, the creatinine-inulin clearance ratio is depressed to 1.1, argues against tubular reabsorption of inulin. [For the preparation of inulin for intravenous administration see Smith *et al.* (169), (170).]

#### TUBULAR EXCRETION

*In vitro* cultures of proximal tubules from a 3½ month human embryo excrete phenol red and orange G into the lumina in conspicuously higher concentrations than that of the dyes in the culture medium (20). The excretion of neutral red by the perfused frog kidney appears to depend to a great extent upon the diffusion of the dye from an alkaline to an acid medium, and not upon the metabolic activity of the tubule cells. There is thus a marked difference between neutral red and phenol red, since excretion of the latter depends upon an active cellular process (22). Indigo-

carminic acid is excreted by the glomeruli of rabbits in proportion to its free concentration in the plasma; the latter, however, is very small and most of the dye is excreted by the tubules (95).

The tubular excretion of phenol red (133), uric acid (156) and creatinine (155) have been demonstrated in the chicken; of creatinine in the anthropoid apes (168), of iopax, neo-iopax and skiodan in man (171).

One of the most notable advances in the problem of tubular excretion and reabsorption has been the study of the kinetics of these processes. In every instance in which this problem has been examined it has been found that the quantity of solute excreted or reabsorbed by the tubules per unit time increases with increasing plasma concentration until a constant maximal rate is reached which is thereafter maintained as the plasma concentration is raised to higher levels. This has been demonstrated in the dog for phenol red (153), in the chicken for phenol red (133), uric acid (156), and creatinine (155); in man for diodrast and hippuran, iopax (170, 171). The calculation of the maximal rate of tubular excretion of any solute requires knowledge of the rate at which it is being filtered, which in turn requires knowledge of the free or filterable fraction in the plasma. The data required for the calculation of this fraction have been reported in the above papers. [See also Smith & Smith (172).] A maximal rate of tubular excretion appears to be the essential limitation in the excretion of phenol red by the aglomerular tubules of the toadfish and the goosefish (157); satisfactory examination of the excretion of creatinine by the toadfish is made difficult by the circumstance that the rate of tubular excretion of this substance in the aglomerular fish is related to the rate of urine formation (158). It is because of the existence of this maximal rate of excretion that the clearance of the solute (or the inulin clearance ratio) is depressed as the plasma level of the solute is elevated.

That a mechanism of tubular excretion may be shared by certain solutes is demonstrated by the fact that in their simultaneous excretion the elevation of the plasma level of one solute can depress the rate of tubular excretion of the other. This has been shown in man in the effect of diodrast, hippuran, iopax, neo-iopax and skiodan upon the excretion of phenol red (170, 171) and in the effect of phenol red upon the excretion of diodrast (170).

The available data on tubular excretion and reabsorption have

been summarized by Shannon (161), who has applied the mass law to the kinetics of tubular activity.

#### CREATININE

The identity of the chromogenic substance in human plasma giving the Jaffe reaction and the mechanism of its excretion continue under debate. Whatever the final answer to this problem, it is clear that the creatinine to inulin ratio in man is highly variable and that neither the endogenous nor the exogenous creatinine clearance can be taken as an accurate index of the rate of glomerular filtration, from which they may differ by fifty per cent or more (119, 120, 121, 181, 200, 201).

Popper & Mandel (135) defend endogenous creatinine clearances in man as a measure of glomerular filtration. No new data are offered other than xylose to endogenous creatinine ratios which range from 0.22 to 1.29, and their argument is confused by a complete misreading of the investigations of others. Since the creatinine clearances reported by Popper & Mandel (as well as by many other investigators) are determined on voided urine specimens they are not trustworthy as single clearance periods.

Forster (53) has extended the clearance method to the bullfrog. The inulin and creatinine clearances are identical at all plasma levels of both substances, indicating identity with the glomerular clearance. The creatinine and inulin clearances in the sheep are identical (154). (These clearances differ only in man and the apes, the chicken and the fishes.)

#### UREA AND AMMONIA

Shannon (159) has examined the simultaneous urea-creatinine clearances in normal dogs under conditions of high urine flow induced by the intravenous administration of glucose, sodium sulphate or urea. As the creatinine urine to plasma ratio is reduced from 10 (the minimal value obtainable during water diuresis) towards 1.0, the urea-creatinine-clearance ratio approaches 1.0, indicating that the reabsorption of urea is entirely a passive process due to the diffusion gradient between urine and blood created by the reabsorption of water. There appear to be two phases in this reabsorptive process; one is related to the obligatory reabsorption of water in the proximal tubule [see Smith (167), p. 230], and accounts for the deficit in the urea clearance at the

highest urine flow obtainable under water diuresis; as the reabsorption of water is blocked by a substance such as sodium sulfate the urea clearance approaches the creatinine clearance. The further deficit in the urea clearance associated with low urine flows is attributed to diffusion in the distal portions of the nephron. The same description may be applied to normal man and to subjects with glomerulonephritis. For any given degree of concentration of the glomerular filtrate (inulin urine to plasma ratio) the reabsorption of urea proceeds in the nephritic kidney essentially as it would in the normal kidney. Since the capacity to reabsorb water is impaired by disease, the fraction of urea reabsorbed decreases as the inulin (or urea) clearance decreases, so that the urea clearance in advanced nephritis approaches the rate of glomerular filtration (24).

Chesley (25) has criticized the calculation of the "standard" urea clearance ( $U\sqrt{V}/B$ ) on the grounds that below a critical urine volume (0.35 cc. per minute) this formula gives erroneously low results. At low urine volumes the urine to blood ratio of urea appears to be maximal and constant in any one individual, and can be related directly to the average normal value of 91.5. (Chesley has called this maximal ratio a "minimal" clearance, but this term should perhaps be reserved for literal use; it would be better, in observations at urine flows below 0.35 cc., to speak of the "maximal urea urine to blood ratio.") The validity of the maximal urea urine to blood ratio was tested by 241 observations on normal, nephritic and toxemic subjects for whom control clearances were available at urine flows above 0.35 cc. per minute (26).

When the urine flow in normal, pre-eclamptic and nephritic subjects is reduced by abstinence from food and water to 0.35 to 0.50 cc. per minute, urea, endogenous creatinine, phosphate, total nitrogen and total solids become maximally concentrated in the urine, presumably due in part to the fact that the urine has reached a "concentration ceiling." However, the urine flow may be considerably reduced below this critical volume (down to 0.05 cc. per minute), under which conditions  $UV$  for all the substances named varies directly as  $V$  (since  $U$  is constant). This fact, and the fact that the endogenous creatinine clearance varies directly in proportion to  $V$ , are interpreted as indicating that in oliguria  $V$  is directly proportional to the rate of glomerular filtration, the latter being reduced considerably below normal levels (27). (It is



rather more than possible that the constancy of  $U$  in the case of urea and electrolytes is due to reabsorption, and this possibility is not certainly excluded in the case of endogenous creatinine. A final decision, therefore, on the important question of the mechanism of oliguria should perhaps be deferred until reabsorption has been definitely excluded.)

The relative values of the arterio-venous extraction ratio of creatinine and urea in the dog are unchanged from the normal after the induction of acidosis. The renal blood flow calculated from the extraction ratios and clearances of these two substances agree, both before and during acidosis. These results demonstrate that in the dog urinary ammonia is not formed from urea (3).

The exogenous urea-inulin clearance ratio at all plasma levels of urea in the chicken averages about 0.75, indicating that urea is excreted by filtration and partial reabsorption, as in the mammals (134). The urea-inulin clearance ratio in the sheep averages 0.52 (154).

The urea clearance in the normal rat (milk diet) averages 10.9 cc.  $\pm$  3.1 cc. per sq. m. of body surface per minute (45).

The effect of age upon the urea clearance, the urea nitrogen content of the blood, the concentrating ability of the kidneys and the maximum specific gravity of the urine in normal man have been reported by Lewis & Alving (104).

Comparisons of the creatinine and urea extraction ratios in dogs with explanted kidneys show that marked variations in the tubular reabsorption of urea may occur momentarily which are not evident in urine samples collected over thirty to sixty minutes. Substantially complete reabsorption may occur for short periods. Though complete reabsorption may occur during cessation of filtration in anuria, the spontaneous variations in reabsorption do not seem to be due to momentary anuria, or to ischemia or reflex excitation (73).

#### GLUCOSE, XYLOSE AND SUCROSE

The tubular reabsorption of glucose (dog) is limited by the circumstance that the tubules are able to transfer only a certain maximal quantity from tubular urine to blood per unit time. When the rate of glomerular filtration of glucose is such that glucose is presented to the tubules at less than this maximal rate, reabsorption is essentially complete (162). In certain endocrine disturb-

ances a considerable elevation of blood sugar may occur without glycuresis (32).

Xylose is reabsorbed by the same tubular mechanism as glucose (dog). Elevation of the plasma glucose to a level high enough to load the reabsorptive mechanism to capacity with glucose reduces xylose reabsorption to zero, so that the xylose clearance equals the rate of glomerular filtration (160). Hyperglycemia also raises the xylose-inulin clearance ratio from the normal value of 0.8 to or towards 1.0 in the frog (53). The xylose-inulin clearance ratio in the sheep averages 0.73 (154).

After intravenous administration of sucrose to normal individuals, 89 to 98 per cent of the injected material is recoverable in the urine in twelve to twenty-four hours (88).

In the phlorizinized frog the glucose, xylose and creatinine clearances are identical, but all are below the inulin clearance, indicating that although phlorizin blocks the active reabsorption of glucose and xylose in this animal, it has the effect of altering the tubules so as to permit back diffusion (about twelve per cent) of small molecules (53). [Substantial evidence against such back diffusion in other animals is afforded by the ratio of glucose, xylose and creatinine clearances to the inulin clearance in the phlorizinized dogfish, dog and man (167), chicken (133, 155) and chimpanzee (168).] This drug is known to depress the tubular excretion of creatine and creatinine in the red grouper and the dogfish, and of creatinine in man, and this depression has now been demonstrated for creatinine in the chicken (155) and chimpanzee (168). It apparently impairs the tubular excretion of phenol red in the chicken (133) and it certainly impairs, though it does not completely abolish, this process in man (23). That it is not a general tubular poison, however, is indicated by the fact that it does not significantly impair the tubular reabsorption of chloride or water (167). An important paper on phlorhizin and related compounds has been published by Lambrechts (98).

#### PROTEINS

Babcock (4) has presented evidence that the excretion of albumin and ferric ammonium citrate in rabbits is not a simple process of filtration, for the excretion of both substances is accompanied by swelling of the glomerular epithelium, dilatation of the capillaries and progressive degenerative changes in the tubules.

This observation is important as contraverting previous evidence that the glomerular membranes are normally permeable to proteins having a molecular weight below 70,000. It also throws light on the "threshold" excretion of hemoglobin, which may be due to similar changes in the glomerular membranes. Accepting the implications of Babcock's observations, the upper limit of normal glomerular permeability in mammals remains to be determined. [See Gérard (62).] It is interesting that insulin, normally absent from the urine of rabbits, is excreted after intravenous administration of massive doses (19).

Renal insufficiency in the dog produced by the injection of hemoglobin is due in part to the precipitation of hemoglobin derivatives in the lumina of the tubules, under conditions of urinary acidity and high salt content. The precipitation does not occur if the urine is alkaline (33). Similar precipitation accounts in part for transfusion reactions in man and can possibly be prevented by alkalinizing the urine prior to transfusion (34). [See also Gérard (62).]

#### WATER EXCRETION AND THE PITUITARY GLAND

The subject of diabetes insipidus has been fully reviewed by Fisher, Ingram & Ranson (50), who report the extensive investigations carried out by themselves and their collaborators, and affirm the widely accepted view that experimental diabetes insipidus is due to a deficiency of an hypophyseal antidiuretic hormone. This principle, they conclude, is normally secreted by the *pars nervosa*, the median eminence and the infundibular stem. The *pars nervosa* also elaborates the oxytocic and pressor principles, the latter apparently being identical with the antidiuretic principle. The *pars nervosa* is under the nervous control of the supraoptic nuclei, the integrity of these nuclei and of the supraoptico-hypophyseal tract being necessary for the maintenance of normal water exchange. It is through the supraoptic nuclei that reflex changes in water excretion are mediated. After removal of the posterior lobe in the dog, cat, monkey and rat a transient phase of polyuria is usually followed by an interphase of nearly normal water exchange, before permanent polyuria develops. In both the transient and permanent phases of diabetes polyuria is primary to polydipsia, but water deprivation may be tolerated for a considerable time (chronic water deficit) without harm except in extreme in-

stances, indicating that some mechanism other than the supra-optico-hypophyseal system is operating to prevent undue water loss (51). As first demonstrated by Richter, the presence of the anterior lobe is necessary for the appearance of the permanent phase, for which circumstance, Fisher, Ingram & Ranson remark that the general depression of metabolism and activity which follow removal of the anterior lobe may be sufficient explanation. As one specific factor they point to the atrophy of the adrenal cortex, a suggestion which seems to hold considerable promise. (See section on adrenal cortex.)

In a preliminary note Keller (90) reports that the anterior lobe is not essential for the occurrence of diabetes insipidus in the dog, but its presence greatly intensifies the effects of a deficiency of the antidiuretic principle. A small remnant of antidiuretic tissue is able to regulate the water exchange after complete anterior lobectomy, but inadequate to meet additional demands. The cessation of the transient phase of polyuria is not due to a temporary depression of anterior lobe activity (91).

Removal of the posterior lobe in the rat may be followed by prolonged polyuria even in the absence of the anterior lobe, a transitory polyuria usually supervening before the permanent phase develops, either with or without removal of the anterior lobe. But removal of the anterior lobe in the cat checks permanent polyuria (37). Hypophysectomized rats that do not develop permanent polyuria differ from hypophysectomized dogs, in that they show no increase in urine flow when given anterior lobe extract, with or without thyroid (193).

The rate of glomerular filtration (creatinine clearance) is unchanged during the transient phase of diabetes insipidus in the dog, while the reabsorption of urea is somewhat diminished, probably because of diuresis. Both processes remain normal during the interphase. Soon after the onset of permanent polyuria, the filtration rate falls and the degree of urea reabsorption is substantially reduced. After prolonged permanent polyuria the filtration rate returns towards normal. Anterior lobe extract raises the filtration rate and decreases reabsorption of urea in normal dogs. The maximal concentrating power of the kidney is substantially reduced in both hypophysectomized and diabetes insipidus dogs (197).

There is no significant difference between the urea clearance in normal cats and in cats with diabetes insipidus. Pitressin re-

duces the polyuria in cats, but not the normal urine flow. (This last result is perhaps due to the circumstance that the urine is normally maximally concentrated.) Anterior lobe suspension induces a considerable increase in diuresis in polyuric cats, though not in normals, but it increases the urea clearance in both (46). Postlobin-O is reported to increase urine output in hydrated and dehydrated rats, and to antagonize the antidiuretic action of postlobin-V (55). But in a note Frazer quotes Ellsworth to the effect that the oxytocic principle has no "diuretic" action in dogs with bladder fistula. The melanophoric principle has no more antidiuretic activity than can be accounted for by the contaminating antidiuretic principle (54). Pitressin reduces the urine flow in the opossum (unless the urine is already maximally concentrated) and increases the excretion of chloride [see also Unna & Walterskirchen (188) and Smith (167) p. 213] and urea.

White & Findley (196) confirm the observation that there is a lag in time between maximal blood dilution (viscosity and conductivity) and diuresis in man; however, they demonstrate a similar lag between maximal blood chloride and chloride excretion, although no lag is evident in the excretion of creatinine. They propose that the lag is characteristic of "threshold" substances only, and attribute it to delay in reactivity of the tubules. If a second water diuresis in man is superimposed upon a first diuretic period, the second diuresis shows about the same lag as the first (194). This lag is present and has a normal value in dogs after removal of the entire hypophysis or of the posterior lobe (197). These results argue against the belief that the lag is due in part to the interval required for the antidiuretic principle in the blood and kidneys to fall to a level at which water excretion will be increased, as posited by Verney and his coworkers.

The inhibition of water diuresis in emotional stress and exercise is attributed to an increased secretion of the antidiuretic hormone (145b). Acetylcholine given intravenously to atropinized dogs inhibits water diuresis, indicating release of the antidiuretic hormone (131). Observations interpreted as demonstrating the urinary excretion of the antidiuretic principle in rats or cats have been reported by Gilman & Goodman (66) and others (16, 85) and the antidiuretic principle is reported in the urine of a subject with hyperfunction of the posterior lobe (127). But Walker (191) reports failure to find the antidiuretic principle in the blood or

urine of either hydrated or dehydrated rats and rabbits. The antidiuretic activity of shed blood has been attributed to histamine (68); an antidiuretic principle is present as a contaminant in heparin (192), and an antidiuretic colloid is present in urine which apparently is not the pituitary principle (191).

Morphine and phenobarbital exert a marked antidiuretic effect not only in normal dogs, but also after hypophysectomy and after inactivation of the adrenal and denervation of the liver. It is concluded that neither the pituitary nor the adrenal medulla are involved in the antidiuretic action of these anesthetics (13), the belief that the oliguria of anesthesia is a pituitary-release phenomenon being thus questioned.

Renal damage and functional derangement may readily be elicited in rabbits by the injection into the renal artery of relatively small doses of the pressor principle of posterior pituitary gland (105).

#### ELECTROLYTE BALANCE

McCance & Widdowson (112, 113) have shown that in experimental salt deficiency in man produced by diet and sweating the extracellular fluid (measured by sucrose or inulin) may be reduced by 28 to 38 per cent. In spite of the fact that the blood pressure is well maintained, there is a marked reduction in the inulin, creatinine, sucrose and urea clearances. At equal urine volumes the urea clearance is excessively reduced, probably due to prolongation of the time of contact of the urine with the tubules at a reduced filtration rate (159), and consequently there is considerable urea retention. During salt deficiency diuresis to ingested water is considerably reduced.

In uncompensated alkalosis in man the rate of filtration may be very low, and this circumstance, accompanied by an excessive reduction in the urea clearance, leads to urea retention. The urea, creatinine and inulin clearances rise after correction of the alkalosis. Data are given on phosphate, chloride and sodium clearances (114). (It is interesting that during the period of reduced inulin clearance the potassium clearance exceeded the inulin clearance by 25 per cent, suggesting the possibility of the tubular excretion of potassium in man.)

The maximal concentration of sodium chloride in the urine of the dog is constant and approximates 1.8 per cent, while that of urea is 9.0 per cent. When hypertonic sodium chloride and urea

are administered simultaneously, the presence of high concentrations of urea in the urine has no effect on the maximal concentration of sodium chloride. Mercurial diuretics (which are reported to increase the excretion of sodium chloride) do not lower the maximal concentration of sodium chloride (67).

#### ADRENAL CORTEX

Although the function of the cortical hormone is still a matter of vigorous debate, it is known that it decreases the urinary excretion of sodium and chloride, and increases the excretion of potassium, in adrenalectomized animals, in subjects with Addison's disease, and in normal dogs and men (203). Previous investigators have pointed to the kidney as at least one important site of action, but the specific effect of the cortical hormone on renal function is clearly indicated by Harrison & Darrow (79, and personal communication), who first report renal clearances in this problem. In adrenal insufficiency in the dog the potassium clearance is decreased, due to the fact that the maximal urine to plasma ratio of potassium is reduced to about 10, in contrast to 40 to 75 in the normal. It is not determined why the ability of the tubules to concentrate potassium is thus impaired, but the resulting decreased potassium clearance is adequate to account for the potassium retention. On the other hand, the sodium clearance is increased, due to the failure of tubular reabsorption. Since electrolytes in general, and sodium chloride specifically, cannot be concentrated in the urine beyond a certain "concentration ceiling," the increased sodium clearance leads to increased water excretion. (See previous section, and Smith (167), pp. 203-6.) Loss of electrolytes and water reduces the volume of the extracellular fluid, which is further depleted by movement of extracellular water into the intracellular compartment in consequence of excessive salt depletion in the former. The resulting oligemia leads to reduction in the rate of glomerular filtration, and this circumstance, coupled with oliguria, leads to urea retention. The symptomatic relief afforded by sodium administration appears to be explicable in terms of the temporary restoration of extracellular fluid volume. It would appear that the water-electrolyte, azotemic and perhaps the circulatory features of the insufficiency syndrome can be explained by the specific action of cortical hormone on the renal tubules.



In line with this explanation, it is noteworthy that Margitay-Becht & Gömöri (109) report that the exogenous creatinine clearance is reduced in Addison's disease, a circumstance which they explain in terms of the co-existent hypotension, dehydration and rise in colloid osmotic pressure. Their attribution of azotemia to the decreased filtration rate is correct in principle, though they are unaware of the importance of oliguria as an additional factor.

Silvette & Britton (164) report that cortical extracts administered to (1) normal or (2) adrenalectomized opossums concurrently receiving (a) water, (b) saline or (c) urea-saline by mouth increased the excretion of water and decreased the concentration of chloride and urea in the urine. On the basis of these and other observations (163) they advance the important hypothesis that "the diuretic hormone of the adrenal cortex acts in physiological antagonism to the antidiuretic hormone of the posterior pituitary lobe." (Examining the data presented by Silvette and Britton, the reviewer notes that in all instances except 1a and 2a the urinary chloride is close to the "concentration ceiling" (0.65 to 1.0 per cent) as revealed in both (1) and (2) by the administration of antidiuretic hormone, and consequently the change in urine flow under cortical extract may reflect nothing but the fact that the extract has in their experiments invariably increased the excretion of chloride, and sometimes of urea. The increased excretion of chloride may be due to increased filtration rate, to an increased excretion of potassium or to the paradoxical decreased reabsorption of sodium which is characteristic of this species. The single exception to this explanation is in 2a, but here the increased water excretion after cortical extract may be explained by a restitution of glomerular filtration, as demonstrated by Harrison & Darrow. Without knowledge of the filtration rate and of potassium and sodium clearances, the data cannot be interpreted with certainty.)<sup>1</sup>

<sup>1</sup> Oliguria may obtain in any condition where hemoconcentration, circulatory inadequacy, hypotension or other systemic change such as would work against glomerular filtration is present; conversely, an increase in urine flow may result from reciprocal systemic changes, or from an increased excretion of electrolytes, urea, glucose, etc. It would appear to be inadvisable to speak of any circumstance or factor that merely increases urine flow as a "diuretic hormone" until specific action in accelerating the excretion of water is demonstrated. It may be noted here that the excitation or inhibition of the sensitive supraoptico-hypophyseal mechanism and the possibility of variations in the secretion of the antidiuretic hormone are neglected by many observers in discussing the "diuretic" action of hormones, drugs, etc.

The effectiveness of cortical extracts and crystalline cortical derivatives in maintaining adrenalectomized dogs parallels their effectiveness in causing renal retention of sodium and chloride and in promoting the excretion of potassium. The synthetic compound, desoxycorticosterone, can be substituted successfully for cortical extracts (182).

In adrenalectomized rats there is an increased excretion of sodium and chloride, but a questionable increase in potassium excretion (147). When kept on a life-sustaining diet these animals show a urine flow approximately five times the normal, but when the diet is changed to a non-sustaining diet the urine flow drops below normal (7). Diuresis in the rat is present apparently only in the milder phases of adrenal insufficiency; in the acute phases the urine flow may be below normal levels, though water output always tends to exceed intake. Theelin is toxic to adrenalectomized rats and produces a conspicuous diuresis (59).

Adrenalectomy in cats with diabetes insipidus produces the same external symptoms and the same rise in the serum potassium concentration as in normal cats, but the serum sodium and chloride do not decrease consistently as in the controls, indicating that changes in sodium are less characteristic of adrenal insufficiency than are disturbances of potassium metabolism or excretion (202). The double operation results in a rapid decline in fluid exchange, the fluid intake falling more than the urine output and leading to a negative water balance. The reduction in fluid intake is primary. Water balance with polyuria can be reestablished by cortical extracts (86).

#### PATHOLOGY

The rate of excretion of cyanol and azofuchsin after standard doses in rabbits has been compared with the rate of excretion of inulin and phenol red by Ehrich, Bartol & Wolf (43). On the basis of the data so obtained these investigators utilize cyanol and azofuchsin excretion as tests of glomerular or tubular damage in rabbits treated with nephrotoxins (44). (Though this method of investigation has perhaps some qualitative value, it is clearly incapable of giving reliable quantitative results. Although recognizing the difficulty of applying the clearance method to rabbits, the writer feels that it is unfortunate to proceed in pathological investigations without full knowledge of plasma concentration,

protein binding, and rate of glomerular filtration in each specific observation.)

Tartrate nephrosis in the dog is accompanied by a decrease in the urea, xylose, inulin, creatinine, and phenol red clearances. The appearance of ferrocyanide in the cells of the proximal tubule indicates that the permeability of these cells has been increased, and is advanced as evidence that the reduction in clearances is due to back diffusion. (But for a contrary argument see the first section of this review.) Evident injury is limited to the proximal tubule (126).

#### RENAL BLOOD FLOW

The important subject of renal ischemia and its relation to essential hypertension has been reviewed by Goldblatt (70) and Blalock & Levy (11). Constriction of the renal artery or of the ureter in the unanesthetized dog sufficient to induce hypertension produces a permanent reduction in blood flow as measured by a venous cannula (100, 102). Removal of one kidney is followed by a slowly progressing increase in the blood flow of the remaining kidney until after three months it is nearly equal to that of the two kidneys (101). In all instances oxygen consumption parallels blood flow, there usually being very little change in arterio-venous differences. In a kidney transplanted to the carotid artery reduction in renal blood pressure effected by compression of the proximal carotid is followed immediately by a reduction in both renal blood flow and the rate of glomerular filtration (103).

Constriction of the renal artery sufficient to produce hypertension in the unanesthetized dog does not necessarily alter the urea, creatinine, inulin and phenol red clearances, although a temporary decrease in the phenol red clearance usually occurs during the twenty-four hours after operation (31).

When the renal artery of the anesthetized dog is constricted the renal blood flow decreases (as measured by the thermostromuhr method), only to recover in a short time almost to its previous level without a change in systemic blood pressure. As many as four increases in the degree of constriction may be followed by successively diminishing rises in blood flow. Intrarenal, vascular reflex dilatation is considered responsible for this phenomenon (152), which has also been noted in anesthetized, laparotomized dogs (42).

Rats which become hypertensive several months after subtotal nephrectomy do not have renal ischemia; the flow per gram of kidney (venous outflow) is nineteen per cent less than in rats a few days after subtotal nephrectomy, but the same as that in rats with unilateral nephrectomy without hypertension (36).

There is a marked difference in the abilities of kidneys of different species to adapt themselves to induced ischemia, the rabbit readily developing necrosis (39).

The action of acetyl- $\beta$ -methylcholine, splanchnic stimulation and ergotamine on kidney volume suggests that the renal vessels lack vasodilator fibers, the splanchno-peripheral balance governing the vasomotor tone (1). Histamine reduces the creatinine and urea clearances in man, coincidentally with, but not in proportion to, the reduction in systemic blood pressure (10). Heat produced by electromagnetic induction slightly reduces the urea and creatinine clearances in subjects with renal disease or other affections (12). [It cannot, however, be concluded from such observations that the renal blood flow is unaffected, since the rate of filtration, and therefore the urea clearance, can vary independently of the renal blood flow (23). See below.]

The important subject of pressor and depressor substances extractable from the normal and ischemic kidney cannot be included here for lack of space. However, it may be noted that the renal pressor substance, renin, causes a decreased renal blood flow in anesthetized dogs, apparently by constricting the efferent glomerular arterioles. Tyramine apparently constricts the afferent arterioles (117). The pressor action of renin is enhanced by cocaine and inhibited by ergotamine (199).

Epinephrine has no effect on the urine flow in the aglomerular toadfish, but in the glomerular puffer it may increase the urine flow five hundred fold (186).

The rate of urine flow in the frog is related to the rate of glomerular filtration (53), as in the teleost and rabbit, but not in dog or man.

In laparotomized, anesthetized dogs the renal blood flow (as measured by the thermostromuhr method) is to a great degree self-regulating, particularly against changing arterial pressure. Denervation and decapsulation lead to an increase in renal blood flow, which is decreased by epinephrine and increased by histamine; the kidney does not participate in the vasoconstriction

elicited by the carotid sinus reflexes, and does not show a true "reactive hyperemia." Pituitrin reduces the renal blood flow in the oliguric state but has no effect during diuresis (80, 130, 150, 151, 174, 175, 179, 187). (The unphysiological conditions under which the above observations were made detracts seriously from their otherwise great value.)

Mann *et al.* (108) report figures showing that a great reduction in the diameter or area of the lumen of a blood vessel is required to produce any marked reduction in blood flow. It would seem that this would be true only where the flow is primarily conditioned by a resistance beyond the point of constriction, as in the above experiments, but the authors do not mention this important circumstance.

The errors in the thermostromuhr method have been more accurately evaluated by Barcroft & Loughridge (6).

The diodrast clearance has been applied to the measurement of the blood flow to the renal excretory tissue in man. In fifteen normal subjects this averages 1340 cc. of whole blood, or 770 cc. of plasma per minute; the average filtration rate in these subjects (136 cc. per minute) is 17.7 per cent of renal plasma flow (23,170). The action of epinephrine, nitrite, theophylline, typhoid pyrexia and water diuresis on the renal circulation and filtration rate in man has been reported. The renal blood flow in man is apparently controlled by efferent rather than afferent arteriolar tone; the hemodynamics of the glomerular apparatus are such that the blood flow may be cut in half or nearly doubled without appreciable change in the rate of filtration (and therefore in any of the related clearances, such as urea and creatinine).

By raising the plasma diodrast level to a point where the tubules are excreting this substance at a maximal rate a figure is obtained (expressed as mg. of diodrast-iodine per min.) which is proportional to the quantity of intact excretory tissue in the kidneys; when corrected for the quantity of diodrast excreted by filtration, this datum affords an index of the total tubular excretory mass ( $Tm$ ) (170).

In subjects with essential hypertension there may be marked relative renal ischemia (cc. of blood per unit of  $Tm$ ) but whether relatively ischemic or not, nearly all subjects with essential hypertension are characterized by a high filtration fraction, indicating excessive tonus of the efferent glomerular arterioles. The disease

produces profound changes in tubular function and there may be a considerable reduction in  $T_m$  before the rate of glomerular filtration is appreciably decreased (71).

#### RENAL HYPERTROPHY AND DIET

The right kidney of albino rats is three to four per cent heavier than the left, a relationship not modified during the renal hypertrophy induced by high protein diet (106). The compensatory hypertrophy induced by unilateral nephrectomy is increased by an increase in the protein intake, the effect being greater in old than in young rats (107).

The metabolic stimulant, dinitrophenol, causes renal hypertrophy in albino rats in proportion to the quantity of drug ingested, the hypertrophy not being prevented by large doses of Vitamin B<sub>1</sub> (125). No renal hypertrophy is induced by prolonged diuresis in normal rats, though compensatory hypertrophy is apparently accelerated by diuresis in nephrectomized rats (178).

Amino-acids, lactic, glycolic, acetic and propionic acids and deaminated glycine increase the post-prandial urea clearance in dogs. Gluconic acid, which is not metabolized, has no effect (81). The urea and inulin clearances in dogs are decreased in vitamin-A deficiency, though terminally these clearances may rise to normal or supernormal values. Vitamin A can apparently elevate the filtration rate above normal in the dog, and it elevates the urea and creatinine clearances from a subnormal level in human subjects with avitaminosis A (82).

#### NEW CLEARANCE DETERMINATIONS

The sulphanilimide clearance in the dog is 20 to 30 per cent of the creatinine clearance; the clearance ratio is independent of plasma level and increases with increasing urine flow. This clearance in man averages slightly over 20 cc. per minute (110). (Presumably sulphanilimide is reabsorbed by passive diffusion, as is urea, though it is unexplained why it should be reabsorbed to a greater extent than is urea.)

Vitamin C is excreted by filtration with tubular reabsorption (man). As in the case of glucose, the reabsorptive process is limited by a maximal rate characteristic of each subject (140).

The uric acid clearance in man at low plasma levels is about ten per cent of the urea clearance, or six per cent of the creatinine

clearance (17). (Unlike urea and sulphanilimide, the uric acid-creatinine clearance ratio rises rapidly on elevation of the uric acid plasma level, this fact indicating active tubular reabsorption.)

#### MISCELLANEOUS

Brevity requires that the following papers be mentioned only by title: criticism of filtration-reabsorption theory (29); solubility of salts in urine (166); maximal intrapelvic pressure (115); increased ureteral pressure and urine formation (132); anoxia and urine excretion (184); nephrectomy, kidney phosphatase and excretion of phosphate (64); parathormone and phosphate excretion (69); oxygen, carbon dioxide and nitrogen tension of urine (148, 165); histological observations during the excretion of albumen (96) and of urea and chloride (47); diurnal rhythm in water and chloride excretion (63); the effects of the "alarm reaction" on urine flow (87); effects of denervation on vascular responses (116) and excretory activity (75); a diuretic principle in the small intestine (149); absorption from bladder (28); clinical observations on urea clearance (74); variations in the urea to creatinine clearance ratio in health and disease (9); plasma concentration and rate of excretion of endogenous creatinine (49); effects of food, drugs, etc., on endogenous creatinine clearances in man (122, 123, 135, 136, 137, 138); action of large doses of epinephrine on urine flow (185); renal activity and oxygen consumption in relation to temperature (30); organic diuretics (189); absorption and excretion of mercurial diuretics (35, 83, 99); mercuric chloride and phenol red-ferrocyanide excretion in frog and rat (40); osmotic pressure of chicken urine (97); salt diuresis in rats after removal of the posterior lobe of the pituitary (180); response to water in patient with diabetes insipidus surgically produced (195); action of adrenalone and dioxypheylpropanolamine on diuresis (207); diuresis and blood dilution (48); effect of pituitrin, theophylline, etc. on chloride excretion (188); production of anemia by pituitrin (37); chronic reabsorption of urine in dog (60); salt and water metabolism in nephrectomized rats (129); creatinine retention in uremia (14); maximal urine urea concentration after adrenalectomy (176, 177); changes in urinary constituents after glucose and fructose *per os* (5), after exercise (77, 128), during potassium and sodium diuresis (88) and in relation to posture (84); excretion of ammonia by heart-lung-kidney (61); ammonia formation in rela-



tion to titratable acidity (21); renal anoxia and ammonia formation in uremic acidosis (92); glycosuria in uranium nephrosis (118); mechanism of experimental uremia (111); reflex anuria (186a); voluntary water intake and maximal output in diabetes insipidus in various mammals (142); sodium chloride and urea intake and voluntary water consumption (65); potential differences across tubules (198); excretion by grasshopper (18); function of pro- and meso-nephros in *Ammocetes* (183); and a review of comparative physiology of the blood (139).

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DEPARTMENT OF PHYSIOLOGY  
NEW YORK UNIVERSITY COLLEGE OF MEDICINE  
NEW YORK CITY, N. Y.

## GENERAL AND LOCAL ANESTHESIA\*

BY M. H. SEEVERS

*Department of Pharmacology and Toxicology  
University of Wisconsin, Madison*

### GENERAL ANESTHESIA

This review is restricted to publications<sup>1</sup> pertaining to the volatile and gaseous anesthetics and to certain non-volatile compounds which are widely used for basal narcosis or laboratory anesthesia. The literature on the opiates, alcohol, and the barbiturates has been ignored although reference to these substances has been made for comparison. Only work of a fundamental pharmacological nature has been selected from the voluminous experimental and clinical literature on general and local anesthesia. Reviews of the volatile anesthetics (1), divinyl oxide (2), the halogenated hydrocarbons (3), trichloroethylene (4), and the anesthetic gases (1, 5) are available. Guggenheim (6) discusses the relation of chemical constitution of the narcotics to their pharmacological action.

*Theories.*—Meyer (7) and Clark (8) analyze the evidence in support of the rival lipid and adsorption theories of narcosis. Clark states that the theory of adsorption is supported by more data of a quantitative nature, although the evidence does not entirely exclude the lipid theory. Burger (9) finds some correlation of potency of the narcotic hydrocarbons with molecular configuration and postulates adsorption to be influenced by the valency forces exerted according to the Baeyer-Theile theory. An electric theory of anesthesia is proposed by Burge *et al.* (10, 11), based upon their observation that a decreased negative potential of the cerebral cortex exists during narcosis. Spiegel & Spiegel-Adolf (12) interpret their finding that all narcotics decrease conductivity within the central nervous system, to be in support of the theory that cellular permeability for electrolytes is diminished during narcosis. Jurišić (13) believes that narcotics produce a sol→gel transformation of the living substance.

*Central nervous system.*—Investigators are agreed that light anesthesia with tribromoethanol and the barbiturates produces minor

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changes in the action potentials of the cerebral cortex similar to those of sleep (14, 15, 16). Spontaneous waves are infrequent in deep anesthesia with these agents (14). Ether and chloroform produce a marked decrease in both voltage and frequency (14, 16). The large cortical response to sciatic stimulation during anesthesia with the barbiturates or tribromoethanol and the absence of response during ether anesthesia suggests to Derbyshire *et al.* (14) that the former compounds suppress cortical activity without blocking pathways to it, whereas ether blocks the lower pathways before the cortical pathways are eliminated. Bremer (17) finds that ether weakens the sensory reaction of the acoustic cortex to sounds both of low and high frequencies, whereas the barbiturates, as also sleep, eliminate only the reaction to high frequencies.

In view of Bard's finding that the frontal lobe plays a definite rôle in postural reactions, Dworkin, Bourne & Raginsky (18) believe that the successive stages in narcosis—ataxia, loss of intercalated reflexes, loss of differentiation, and loss of positive reflexes—are due to a progressive action on the cortical centers. Species variation in reflex excitability is indicated by the observation of Allen (19) that the olfactory respiratory reflex is abolished by first stage ether anesthesia in the dog, and third stage in the rabbit. The polypneic panting noted by Magoun (20) in cats during recovery from ether and urethane seems to be a species characteristic.

Recent studies of brain respiration during narcosis have revived interest in the theory that an inhibition of oxidations in nerve cells is responsible for narcosis. Reviews of work prior to 1937 are available (21, 22, 23). It is generally agreed that fairly high concentrations of narcotics placed in direct contact with surviving brain tissue diminish the oxygen uptake and reduce oxidations (24, 25, 26). Conflicting evidence and opinion exist concerning proper methods of study, reversibility of action, the *in vitro* effects of drugs in concentrations which are comparable to those required for narcosis *in vivo*, and the oxidation capacities of brain tissue from narcotized animals.

Quastel & Wheatley (27) were able to demonstrate reversibility by washing brain slices with phosphate-saline-glucose medium following the inhibition produced by chloretone and other crystalline narcotics. They found that the inhibition follows a sigmoid curve; the concentration of the substrate is unimportant; ions such as potassium and calcium modify the effect, and differ-

ent agents inhibit the oxidation of some substrates more than others (28).

Emerson (29) is unable to correlate the decrease in "autoxidation" which he observed in minced rat brain with depth of anesthesia. He notes inhibitions of similar magnitude in epinephrine-treated animals and finds that the blood-sugar curves of anesthetized animals bear an inverse relationship to the rate of "autoxidation" of their brain tissue. Inactivation of lactic acid dehydrogenase, or of luciferase from the lampbrush, is not obtained with sublethal concentrations of the volatile and gaseous anesthetics (30).

According to Galli & Radici (31), dehydrogenation processes in the surviving brain, lung, liver and kidney of the rabbit are not significantly affected by the volatile or gaseous anesthetics. Lindwall (32) finds nitrous oxide in narcotic concentrations to have no influence on oxygen uptake or on the anaërobic phase of respiration. Tribromoethanol in high dilution reduces dehydrogenation processes in all tissues *in vitro* although oxidations by the brains of animals killed quickly after massive doses are only slightly modified (33).

Using more refined methods (34), Jowett & Quastel (35) determined that the action of ether on sliced cerebral cortex is to inhibit the oxidation of glucose, fructose, lactate, pyruvate, and glutamate, but not of succinate. The inhibition, if it occurs, is progressive and develops as a function of time and of ether concentration. Small inhibitory effects are not reversible although further inhibition is arrested if the ether is removed. With high concentrations, the inhibition is progressive. Respiration of brain tissue from rats previously anesthetized is not measurably affected. The inhibitory effects of ether at anesthetic concentrations are within the experimental error of ten per cent.

In a recent paper, Jowett (36) divides the narcotic agents into two classes with respect to their action on the oxidation of glucose by brain tissue. The inhibitions produced by urethane, magnesium, chloral, phenobarbital, evipal, chloretone, and tribromoethanol, if they do not exceed 40 per cent, vary little with time. The findings indicate that the narcotic reaches a mass-action equilibrium with some component of the respiratory system, so that the inhibition is represented by a simple mass-action equation. The inhibitions which these agents produce are measurable at concentra-

tions of the same order as those which produce general narcosis in the intact animal. Inhibition of oxidation of glucose may therefore be the cause of the narcosis. On the other hand, alcohol and ether inhibit cell respiration progressively and probably ultimately irreversibly. The anesthetic concentrations in this instance are only threshold values for the subsequent inhibition of respiration.

Kerr & Antaki (37) find that short or prolonged anesthesia with ether or chloroform does not significantly affect the glycogen and phosphocreatine content of rabbit brain, although there are increases in the levels of free sugar and of lactic acid after chloroform.

Short anesthesia with the volatile or gaseous agents diminishes the amount of reduced glutathione in lung tissue and blood, according to Galli & Mingazzini (38). The quantity is increased in the liver, but the amounts in kidney and brain are variable. Tissues taken twenty-four hours after narcosis, particularly with nitrous oxide, have an increased content.

Peoples (39) observes that a 50 per cent decrease in total oxygen uptake occurs in the rat during deep ether anesthesia even in the presence of adequate oxygen and glucose. He attributes the decreased metabolism to injury of tissues other than those of the central nervous system. Anesthesia of comparable depth with divinyl oxide does not produce such profound effects.

*Autonomic nervous system.*—Knoefel (40) believes that many of the undesirable side-actions of anesthesia with ether and chloroform, particularly those referable to cardiovascular and digestive function, or those which are secondary to disturbances in carbohydrate metabolism, result from epinephrine liberated by an overactive sympathetic nervous system. This sympathetic stimulation is less with ethylene, or cyclopropane, and is prevented by the barbiturates. Emerson (41) supports this view, in the light of his observation that a significant decrease in the epinephrine content of the adrenal glands of the cat occurs after thirty minutes of ether anesthesia. Premedication with the barbiturates prevents this decrease, and it does not occur during anesthesia with divinyl oxide. Further substantiation for this idea is the fact that light anesthesia with almost any agent produces a significant rise in blood sugar and a marked ketonuria, the latter reaching a peak at different times (42). Tournade & Tournade (43) take the opposite view and attribute the differences in cardiovascular response of

the chloralosed as compared with the etherized dog to a paralysis of secretion of epinephrine by ether. The autonomic nervous system is not affected by bromural, adalin, ethyl urethane, or ethyl chloride, according to Moraeus (44), although marked effects on the frog heart and rabbit intestine are obtained with chloralose and paraldehyde in high dilution.

Lerman (45) notes that the action of ether and chloroform, contrary to that of barbital, is to produce a definite hyperglycemia in all stages of anesthesia. Ether produces a greater hyperglycemia in non-diabetic than in controlled diabetic patients, whereas the rise occurring with evipal is insignificant (46). Paraldehyde produces a consistent rise in blood sugar which reaches a higher level in the poorly nourished dog (47). Hrubetz & Blackberg (48) believe that chloroform and several barbiturates depress the glyco-genolytic power of the liver, as indicated by the relatively small effect of epinephrine on the blood-sugar level.

*Blood.*—Essex, Seeley, Higgins & Mann (49) observe that a profound reduction in the size of the spleen occurs in the dog during ether anesthesia, accompanied by marked increases in the concentration of erythrocytes and of hemoglobin in peripheral blood. This blood concentration is abolished by splenectomy. The barbiturates produce the opposite effect (50). Whereas the spleen plays an important rôle in the sequestration of leucocytes in the rat, this does not occur in the rabbit, and splenectomy in both animals does not affect the erythrocyte or hemoglobin level during anesthesia (51, 52). Paraldehyde produces only slight changes in blood concentration (47).

Preliminary experiments by McAllister & Thorn (53) indicate that adrenocortical extract may preserve the normal plasma volume of the etherized dog.

Ether and chloroform increase the sedimentation velocity of erythrocytes (54), whereas it is decreased by morphine, tribromoethanol (55), or electronarcosis.

A seventy per cent decrease in blood platelets is observed thirty minutes after narcosis with tribromoethanol (56).

Ether anesthesia in the guinea pig and dog is accompanied by an immediate fall in serum potassium, the level being lower than normal for five hours after anesthesia (57).

Biasini (58) finds the blood bromide to be reduced during the administration of ether and chloroform.

An increase in the blood ammonia of ten to fifty per cent is observed during ether narcosis (59) although the urea-nitrogen level remains essentially unchanged (60).

The hydrogen-ion concentration of venous blood is increased during ether induction in the rabbit, moderately reduced during anesthesia, and greatly reduced at death (61).

*Respiration.*—An increase in the oxygen unsaturation and capacity of arterial blood, and a diminished arterial-venous oxygen difference, occur in dogs anesthetized with ether by the cone-drop method (62).

Eastman (63) reports that the oxygen saturation of fetal blood at the time of delivery is not appreciably affected by chloroform or ether. However, five minutes of anesthesia with 90 per cent nitrous oxide results in profound asphyxia neonatorum in 35 per cent of infants.

The old observation that anoxia acts in an additive manner to supplement anesthesia is reaffirmed for analgesic concentrations of nitrous oxide (64).

Courville (65, 66) presents good evidence that the destruction of the cerebral cortex during nitrous oxide anesthesia is invariably due to anoxemia accompanying anesthesia and discounts the statement of Lowenberg, Waggoner & Zbinden (67) that nitrous oxide specifically injures nerve tissue.

Several investigators have studied the action of anesthetics on the respiratory tract. Ernst (68) finds that ciliary movement in the trachea and bronchi is greatly reduced by all the volatile and non-volatile agents except nitrous oxide. The mucus secreted by the frog trachea and esophagus is "stickier" after ether than after chloroform, ethylene, or nitrous oxide (69). Margaritis (70) observes that leucocytes collect on the surface of the tracheal mucous membrane of guinea pigs during chloroform anesthesia. If streptococci are injected intratracheally at this time the superficial cell layers are shed, and the passage of organisms through the epithelium is increased after thirty minutes. Dogs "gassed" with phosgene and anesthetized twenty-four hours later with ether, chloroform, or evipal (71), usually survive, but complications are more frequent than those occurring in anesthesia with nitrous oxide or cyclopropane (72).

*Heart and circulation.*—Disturbances in impulse formation and transmission in the heart commonly occur during anesthesia.

Kurtz, Bennett & Shapiro (73) report an alteration in rhythm in 79 per cent of 109 clinical cases, with all agents. Many of the arrhythmias are of reflex or extrinsic origin, but certain agents appear to alter the intrinsic conduction mechanism, or render lower foci of impulse formation more irritable (74). Changes in the T-wave may be due to anoxic factors secondary to diminished respiration (75), or coronary constriction (74, 76).

The action of chloroform on the heart has been analyzed by de Somer (77). Levy's observation (78) that chloroform sensitizes the myocardium to epinephrine has been amply confirmed. Guedel & Knoefel (79) indict chloroform and ethyl chloride as producing the greatest increase in cardiac irritability, although ventricular fibrillation may occur in anesthesia with any agent.

The thorough study of Meek, Hathaway & Orth (80) indicates that the stimulating effect on the automatic tissues of the dog's heart, as measured by the appearance of ventricular tachycardia after a standard dose of epinephrine, increases in the following order: ether, chloroform, cyclopropane. They point out that the dog heart is less sensitive to chloroform than the cat heart. This fact may account for the variation in results previously reported.

Although anoxia does increase the incidence of arrhythmia with cyclopropane (81), it is difficult to harmonize completely the work of Meek *et al.* (80) with that of Robbins & Baxter (75), who attribute the arrhythmias with cyclopropane to anoxemia resulting from deficient respiration.

Velluda & Russu (82) report that bilateral extirpation of the suprarenal capsules of the dog prevents the production of the epinephrine-chloroform syncope, although removal of the medullary portions is without effect. They also note that attempted reticulo-endothelial blockade with a carmine-lithium hydroxide mixture lowers the incidence of syncope (83). Shen & Simon (84) confirm Hermann & Jourdan (85) in their observation that novocaine like cocaine, if administered with the epinephrine, protects against fibrillation during light chloroform anesthesia. Shen (86) has recently observed that yohimbine, and certain derivatives of dioxane, are effective, and believes that any substance which prevents the pressor action of epinephrine will combat syncope during chloroform anesthesia.

Van Dongen (87, 88) states that ether increases the irritability

of the heart for stimuli which cause fibrillation although it has no effect on heterotopic rhythms caused by epinephrine or barium chloride.

Betlach (89) finds that ether causes negative T-waves from the dog heart to become positive in all of the conventional electrocardiographic leads, cyclopropane in II and III only. Cyclopropane causes a marked deepening of the T-wave in lead IV, while ether is without effect.

Robbins & Baxter (90) find the cardiac output of the dog increased in cyclopropane anesthesia of moderate depth. During very deep anesthesia the output is normal or decreased.

The velocity of the peripheral circulation is diminished after prolonged ether or chloroform administration (91).

The more rapid onset of shock in the etherized, when compared with the amyralized dog, is attributed by Seeley, Essex & Mann (92) to greater fluid loss in the former.

*Muscle.*—Sleeth & Van Liere (93) find the percentage delay in emptying time of the dog stomach with different agents to be: chloroform, 64; ether, 40; nitrous oxide (and anoxia), 15; ethylene cyclopropane, divinyl oxide, 7.

Ether inhibits the motility of the small intestine of the rat during, and for two hours following, anesthesia (94). Burstein (95) finds a similar action of ether on the dog. Cyclopropane inhibits contraction but tone is maintained to fairly deep surgical anesthesia.

Micturition in the cat during induction with ether results from the strong vesicular contractions, the pressure in the bladder increasing with the depth of anesthesia. These effects occur even after denervation of the abdominal wall (96).

Gladish & Elder (97) report that nitrous oxide, ethylene, and cyclopropane do not interfere with the activity of the intact uterus. Paraldehyde alone, or with benzyl alcohol, gives similar results (98).

Richter (99) notes an increase in the reaction time of skeletal muscle of the rat after repeated exposure to ether.

*Liver function.*—The reticulo-endothelial system of the liver is more sensitive to anesthetics than the other structures of this organ, according to Gagliardi (100). He finds an intense but transient insufficiency of the liver, as indicated by the rose bengal test and by the presence of bilirubinemia. The observation that healthy



individuals remain in a state of latent hypercholeemia following ether anesthesia indicates to Jeanneney & Planques (101) that little liver injury has been produced.

The secretion of bile salts is decreased after short exposures to chloroform (102).

Chloroform injury to the liver does not prevent the formation of hemoglobin or urea by this organ, according to Daft, Robschey-Robbins & Whipple (103). The increase in urinary creatine observed by these authors leads them to suggest that muscle injury occurs from circulating toxins of hepatic origin.

Smith, Warner & Brinkhous (104) ascribe the bleeding tendency in acute chloroform intoxication to failure of prothrombin formation by the injured liver.

Goldschmidt, Ravdin & Lucké (105) claim that inhalation of oxygen protects the liver against the necrotizing effect of chloroform and divinyl oxide.

An active liver concentrate which protects against liver necrosis from chloroform and carbon tetrachloride has been obtained by Forbes, Neale & Scherer (106). Neale & Winter (107) determine the active principle to be sodium xanthine. Other purines—nucleic acid, guanosine, guanine, hypo-xanthine and uric acid—give some protection; their oxidation products, allantoin and alloxan, increase the pathogenicity of these hepatotoxic compounds.

*Urinary and tissue constituents.*—The steady increase in uric acid excretion after repeated exposure to cyclopropane is ascribed by Greisheimer, Hafkesbring & Magalhaes (108) to liver injury. These authors find the urea clearance of the dog thirty per cent above the control values after cyclopropane administration.

Ether, and nitrous oxide even more so, increase the urinary excretion of porphyrin (109).

Although ether reduces intestinal putrefaction in the rat intestine, Emerson (110) finds no regular changes in the urinary excretion of indican, urobilinogen, ethereal sulfates, or of free and conjugated phenols by patients anesthetized with ether and other agents.

Ether, chloroform, paraldehyde, and numal produce an increased excretion of oxalic acid in the bile and urine. Borgström (111) indicts anoxia as a causative factor since oxygen inhalation prevents biliary excretion of oxalates after paraldehyde.

Bowman & Muntwyler (112) confirm their previous obser-

vation that a ten- to fifteen-fold increase in the urinary excretion of ascorbic acid occurs following ether anesthesia in the dog, less in rats and guinea pigs. Ecker, Pillemer & Wertheimer (113) find a definite rise in serum ascorbic acid in the guinea pig. The ascorbic acid content of the liver and kidney of the etherized rat is increased (114), but decreased in the adrenals (114, 115). A decrease occurs in all tissues of the guinea pig (114).

An increase in the chloride content of rabbit kidney and lung occurs during ether anesthesia (116).

*Factors influencing anesthesia.*—Ether and acetone are examples of narcotic agents which are more soluble in water than in blood, whereas these solubilities are reversed with ethylene, ethyl chloride, chloroform, and benzene. According to Lazarev (117), a part of the narcotic is present not in true aqueous solution, but "bound" (adsorbed by plasma proteins and erythrocytes, dissolved in fats and lipoids, etc.). He presents evidence (118) to support the concept that weak narcotics are present in aqueous solution in the blood, whereas strong narcotics are completely "bound." He cites the work of Scotti-Foglieni (119), who demonstrated that hemoglobin often binds more of the anesthetic than the lipoids. The concentration of fat in the blood does not affect the distribution ratios of benzene and benzine in blood and inspired air (120). Broussilowska (121) is in general accord with the findings of Lazarev, and attributes the variations in response of different species to differences in their blood constituents, the latter affecting the state of solution of the anesthetic in the blood.

Shtessel (122) presents evidence that binary mixtures of narcotics exert strictly additive effects in producing narcosis in mice rather than acting in a synergistic manner. Only additive effects are noted for hemolysis *in vitro* even with five components, methyl alcohol, ethyl alcohol, ether, chloroform, and acetone.

Tribromoethanol in alcoholic solution has been shown by Macht (123) to be absorbed through the intact skin with sufficient rapidity to effect narcosis in mice. The presence of amylene hydrate increases the rate of such skin absorption.

Neutral fat is reduced both in erythrocytes and plasma, phospholipids in the erythrocytes only, following nitrous oxide-ether anesthesia (124, 125).

Montanus, Ames & Herrmann (126) are unable to establish a typical lipemia curve under ether anesthesia. According to Maruta

(127), chloroform and tribromoethanol produce greater increases in blood cholesterol although similar qualitative changes are produced by all narcotics. Since Livraga (128) finds a reduced quantity of unsaturated fatty acids in blood following ether and chloroform, he postulates a liberation of saturated acids by the solvent action of these agents on tissues.

The intraperitoneal injection of cholesterol hastens and prolongs anesthesia with ether, chloroform, urethane, and barbital. Starkenstein & Weden (129) offer evidence to substantiate the assumption that there occurs a greater transport of these agents to the brain. Intravenous saponin also potentiates narcosis, and is assumed to mobilize body cholesterol, the latter increasing transport. The effects of ether and chloroform, both more lipid-soluble than water-soluble, on the frog heart and isolated intestine, are reduced by the addition of lecithin and cephalin (130).

Konzett (131) reports that the administration of fat-soluble dyestuffs (alizarin blue S, methylene blue, and neutral red) greatly potentiates the narcotic action of paraldehyde, chloral hydrate, and certain barbiturates. Congo red, a fat-insoluble dye, is ineffective.

Ujiié (132) confirms previous observations that liver injury increases the toxicity and prolongs the effects of tribromoethanol and evipal.

Repeated administration of thyroxine to rats lowers their resistance to chloroform but not to ether, according to Sainton & Delauney (133). Tiffeneau & Broun (134) observe that the induction time of anesthesia with propyl bromide is doubled in animals kept at 15°C. when compared with those at 25°C., and they are able to correlate this finding with the concentration of the drug in the brain.

*Methods—Effective concentrations.*—The distribution ratio of ether between air and blood is stated by Robbins (135) to be 1:14.4 at 37°C. He points out that during anesthesia the ether in the inspired air rarely becomes fully equilibrated with the ether in the blood—the average ratio being 1:10. The concentrations of ether in blood, at various levels of anesthesia in the dog, are as follows (mg. per 100 cc.): anesthesia with rigidity, 90; with relaxation, 113 (3.7 to 4.5 per cent ether in inspired air); loss of knee jerk, 143; lid reflex, 150; respiratory arrest, 187 (6.7 to 8 per cent ether in inspired air). For satisfactory anesthesia, 4 to 4.5 per cent

of ether in the inspired air is desirable. He finds no difference in potency with different commercial brands. Cersuni & Broussilowska (136) find a species difference in the distribution coefficients between alveolar air and blood, the values for ether being: 12.9 for the dog; 9.3 for the rabbit. The rabbit also has lower values for benzene and benzine.

Domanski (137) presents a method for the isolation of divinyl oxide from liquids and tissues.

A method for the determination of paraldehyde in tissues is described by Nitzescu, Georgescu & Timus (138) and the following results, expressed in mg. per 100 cc. of tissue, were obtained after the intravenous injection of 0.20 mg. per kg. in the dog: blood, 88; heart, 25; nerve, 11; other tissues, 4 to 8; urine, 8; expired air, 28. Twenty-three per cent of the injected paraldehyde was eliminated in the expired air of the rabbit during a ten-hour period. Paraldehyde is eliminated quantitatively by the lung of the rat (139).

Methods are described for the quantitative analysis of the anesthetic gases in liquids by extraction (140, 141), and for cyclopropane (142) by an iodine-pentoxide method.

Solubility constants for cyclopropane determined by saturation (1), by extraction (143), and by the iodine-pentoxide method (142), are in good agreement. A summary of results is presented in a review by Seevers & Waters (5). At 37.5°C., the solubilities<sup>2</sup> ( $\lambda$ ) are: water, 0.204; oxalated human blood, 0.457; olive oil, 6.99. The solubilities of this gas in cod-liver oil and paraffin oil are similar to that in olive oil (143). The oil-water coefficient is 34.3. The solubility in blood varies with the cell volume and lipid content and is increased after a fat meal (144). Robbins (144) finds the distribution coefficient for air and blood in the dog to be 0.49 *in vivo* and 0.51 *in vitro*, indicating that almost complete equilibrium is attained in this animal. The cyclopropane concentration in right heart blood is practically equal to that in arterial blood within fifteen minutes. Although venous blood still retains some cyclopropane two hours after anesthesia, the major portion of the gas is eliminated within the first ten minutes. Robbins (144) has also determined the concentration of cyclopropane in air and

<sup>2</sup>  $\lambda$ (Ostwald solubility expression) = volume of gas dissolved per unit volume of solvent at a given temperature and for any pressure when the pressure of the gas itself minus the vapor tension of the solvent is equal to the atmospheric pressure.

blood required for various levels of anesthesia. They are in good agreement with results previously published (81).

The analgesia produced by continued inhalation of five per cent cyclopropane in the normal human subject is approximately equivalent to that obtained with forty per cent nitrous oxide or thirty per cent ethylene (145).

Aird (146) and Newman (147) introduce nitrous oxide or ethylene into the cerebral ventricles for encephalography, find the procedure safe, and observe a non-irritating and sedative action.

*Toxicity.*—Ether or alcohol intoxication deprives rabbits of the immunity acquired from the administration of antipneumococcal serum. Since leucocyte emigration to the inoculation site is prevented by these agents, Pickrell (148) considers that the bacteria may proliferate uninterruptedly.

Mengle (149) introduces particulate matter into the peritoneal cavity of dogs and finds that greatest lymphatic absorption occurs with those agents, such as ether, which increase diaphragmatic activity.

Quill (150) presents evidence, from the dog, to discredit the precept that ether anesthesia protects against fatal anaphylaxis in sensitized individuals.

The cat unit of digitalis is greater under dial-urethane (151) or pentobarbital (152) than under ether anesthesia.

The etiology of muscular spasms, or convulsions, which may occur during anesthesia, remains obscure, probably because many factors are involved. Among those cited are specific streptococci (153), alkalosis (154), pyrexia (155), acidosis from hypercapnia and pyrexia (156).

*New agents.*—Molitor (157) has reinvestigated the pharmacological properties of divinyl oxide.

Henderson & MacDonald (158) have examined the actions of methyl, 1,2-dimethyl, and 1,2,3-trimethyl cyclopropanes and find them to be unsuited for anesthesia. The mono- and dichlor derivatives of cyclopropane (159) produce severe pulmonary lesions.

Ethylene oxide, furan, dimethyl furan, tetrahydrofuran, and its dimethyl derivative, have a small margin of safety and produce hepatic degeneration (160, 161).

Anesthesia with *n*-butane, isobutane, *n*-pentane, isopentane, and neopentane is associated with hyperventilation and muscular phenomena, according to Stoughton & Lamson (162).

The anesthetic action of the hexenes and hexanes, both of which may be impurities in propylene (163), is characterized by a small safety margin, although cardiac irregularities are not common.

#### LOCAL ANESTHESIA

Most of the investigations in this field in recent years are of a confirmatory rather than of an original nature. No attempt has been made to review the extensive literature on regional anesthesia. A very satisfactory review is that of Hirschfelder & Bieter (164) in 1932. The relation of pharmacological action to chemical constitution in the 2-alkoxyquinoline derivatives and the alkamine esters of *p*-hydroxybenzoic ethers is discussed by Wojahn (165) and by Rohmann & Scheurle (166).

*Mode of action.*—Reichelt (167) is not able to substantiate the theories of surface anesthesia which postulate retention of the agent in the superficial cell layers of the mucous membrane, since all compounds studied are equally well absorbed from the mucous membrane of the bladder irrespective of their efficiency as local anesthetics.

In view of the fact that washing of isolated nerve readily effects recovery from procaine but not from nupercaine, and chronaxie and rheobase determinations with the two drugs show different characteristics, Régnier & Quevauviller (168, 169) believe that fixation in the nerve cell is dependent upon the chemical constitution of the agent. A like conclusion is reached by Patania (170) based upon the similarity of response of different species to several agents.

Kato (171) finds that the electrical threshold of isolated co-cainized nerve is abrupt rather than progressive during induction and recovery. Impulse transmission is suspended more rapidly if the agent is applied at Ranvier's node.

Rabbeno (172) states that the local anesthetics may be divided into three groups and that the action of the members of each group can be represented by a characteristic time-concentration curve. Combining two agents of different groups often results in potentiation of action.

*Factors affecting anesthetic action.*—The procaine salts derived from combination with phthalic, hippuric and nicotinic acids, are practically devoid of anesthetic action if topically applied, but the action of procaine is intensified fifty to sixty times if combined

with phenyl butyric, undecic, or  $\alpha$ -phenyl valeric acid (173).

Alkaline solutions of local anesthetics produce a shorter and more intense action than the ordinary acid solutions (174).

Stephany & Matschulan (175) find that corneal anesthesia with cocaine is prolonged twenty- to thirty-fold by the previous systemic administration of morphine and simultaneous deposition of fresh egg albumin into the conjunctival sac. Calcium, by vein, likewise potentiates morphine in prolonging cocaine action (176). Matschulan (177) also states that acedicon (a thebaine derivative) prolongs cocaine anesthesia, although thebaine is without activity.

*Methods.*—Bieter, Cunningham, Lenz & McNearney (178) determine the therapeutic ratio  $\frac{(M.L.D.)}{(M.A.D.)}$  of certain drugs for spinal anesthesia in the rabbit to be: metycaine, 4; procaine, 6.6; panthesine, 8; nupercaine, 11.4; tutocaine, 12; pantocaine, 30. They also give data for the duration of action of these derivatives using the same method (179). The duration of action of a 0.25 per cent solution of different agents applied to the isolated sciatic nerve of the frog, is, in increasing order: procaine, metycaine, cocaine, panthesine (180).

Loewe (181) describes a "pseudo-hernia" of the abdomen of guinea pigs after the hypodermic injection of various local anesthetics. The sensitivity of the reaction parallels other methods of assay, although the required dosage is several times greater.

*Factors affecting toxicity.*—Sievers & McIntyre (182) find the toxicity of procaine increased fourfold above that at room temperature, if testing is done at 43°C.

Beutner & Miley (183) observe that calcium reduces the incidence of convulsions from procaine in the guinea pig, and Carratalá (184) describes a similar action, in combination with somnifen, in cocaine poisoning.

Zipf & Hoppe (185) claim that metrazol will detoxify procaine and larocaine.

The toxicity of procaine-epinephrine for the cat is thirty-five per cent lower than that of procaine alone, due to a delay in absorption (186). Abbate (187) also obtains a favorable antidotal action of epinephrine against procaine, pantocaine, and cocaine, but not against nupercaine.

Isenberger & Rice (188) report that ephedrine antagonizes the paralytic effect of procaine on respiration when both are adminis-



tered intracisternally, although ephedrine by vein is ineffective.

Pickering, Steinmeyer & Luckhardt (189) report that solutions of procaine-epinephrine injected into the mental, mandibular and palatine foramina of the dog have a marked pressor action.

Møller & Stefansson (190, 191) offer additional evidence for the potentiation of epinephrine by cocaine, using the blood sugar and ear-vessel response of the rabbit. Cocaine inhibits the catalytic oxidation of epinephrine by tyrosinase *in vitro*, according to Bayer & Wense (192). The capacity of various agents to mobilize sugar in the rabbit roughly parallels their anesthetic efficiency and toxicity (193).

Kronfeld & Lin (194) observe that the protein content of regenerated aqueous humor is much lower after corneal anesthesia with cocaine than with butyn or pantocaine, and attribute this difference to the vasoconstrictor action of cocaine.

The excitability of excised intestinal muscle is temporarily abolished by the local action of cocaine and other agents (195). Kokas & de Ludany (196) account for the observed delay in glucose absorption from the cocaineized jejunum on this basis.

The average quantity of cocaine absorbed from the urethra following the instillation of 15 to 50 mg., as determined by Rupel & Harger (197), is 9.26 mg., this value representing the average quantity unrecovered from urine specimens.

Bullock & MacDonald (198) describe a method, applicable to body fluids, for the determination of the local anesthetics derived from *p*-aminobenzoic acid. The concentration of such drugs after intraspinal administration falls rapidly at the site of injection and only traces may be detected in blood and urine. Bogetti (199) finds an increased urinary excretion of procaine in phosphorus- or mercury-poisoned animals.

Certain new derivatives of quinoline and pyrazoline (200, 201, 202, 203, 204), hydrocotarnine (205), and furan (206) produce an inferior anesthetic action. Other compounds, two derivatives of cytosine (207), two mono-alkylated amino alcohols (208), and several benzoyl derivatives (209, 210, 211, 212, 213) appear to be worthy of clinical trial.

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DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY  
UNIVERSITY OF WISCONSIN  
MADISON, WISCONSIN



## APPLIED PHYSIOLOGY\*

By D. B. DILL

*Fatigue Laboratory, Morgan Hall  
Harvard University, Boston, Massachusetts*

The topics covered under this title will be limited to *muscular exercise*, including fatigue and training, metabolic and respiratory adaptations, blood changes, renal function, industrial physiology, and age; and to the *effects of high temperatures* and of *low and high partial pressures of oxygen*.

### MUSCULAR EXERCISE

*Fatigue and training.*—A method has been developed in Russia for studying changes in muscle in relation to fatigue and training. Fatigue is produced by faradic stimulation for some minutes of one leg of an animal while the other serves as control. If this is repeated for a week or more, various changes take place in the muscle other than the temporary evidences of fatigue. Koldayev & Gelman (1) find that when rabbit muscle is fatigued its ascorbic acid content decreases, but with continued training it increases. In normal rabbit and pigeon muscle the oxidation-reduction potential is +292 to +473 and +49 to +130 mv. respectively. The potential fluctuates in a rhythmic fashion—possibly a phenomenon of biological importance (2). Continuing this study, Chagovetz (3) found in extracts of fatigued muscle and of trained muscle changes in oxidation-reduction potential that point to an accumulation of reduced substances as training proceeds.

Myshkis & Myshkis (4) report that the creatine phosphate of muscle is increased by a protein-free diet and is diminished by a meat diet. If confirmed, this might have an important bearing on the dietary regime of athletes. In this connection, Strieck (5) reports the case of a man who lived for years on a diet that provided a daily intake of about thirty grams of protein. Physical efficiency was high and severe exercise could be carried out without increasing the nitrogen excretion. When one hind limb of each of five rabbits was trained by faradic stimulation muscle hypertrophy was evident and the oxidase content was significantly increased (6). It is suggested that the increased oxygen utilization in the

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blood of the trained athlete depends not only on this increase of catalyzers but also on structural adaptations in his musculature.

Schroll (7) finds that training dogs to run on a treadmill increases the capacity of heart, liver, kidneys, and skeletal muscle for oxidizing ascorbic acid. Dehydroascorbic acid is more readily reduced, particularly by skeletal muscle. When guinea pigs are deprived of ascorbic acid, Ratsimamanga (8) finds that their muscle contains more lactic acid than normally and fatigues rapidly. Administration of ascorbic acid increases liver and muscle glycogen; with the addition of cortin, recovery is even more rapid. These experiments agree with those of Brack (9); the injection of cortin delays the onset of fatigue in an isolated muscle by two hours. When ascorbic acid is injected also, the results are even more favorable.

Three co-ordinated studies of the rôle of the cortico-adrenal hormone in relation to fatigue have been carried out in the past year (10, 11, 12). Spontaneous activity in the white rat was unaffected by various commercial preparations. The Hartman extract had no effect on basal metabolism of man but there was usually a reduction in oxygen consumption in moderate work. It did not modify the capacity of man for severe anaërobic work.

Fatigue is thought to deplete the epinephrine content of the adrenals, but Syozi (13) does not agree. In rats, whether normal or with denervated adrenals, exhaustion did not change the hormone content of the adrenal medulla. Removing the adrenal medulla of young rats did not modify their weight gain or work performance (14). Adaptation to muscular exercise and cold is not lost when rats are adrenalectomized if sodium chloride is supplied. Furthermore, adaptations to these stresses can be accomplished after adrenalectomy. Blood sugar regulation improves with adaptation; adrenals are not essential for this role (15). On the other hand Meythaler (16) concludes that the sympatho-adrenal system limits the level of performance in highly trained athletes.

A standard work experiment (17) carried out before and after a bicycle ride of 100 to 150 km. showed that fatigue increased pulmonary ventilation, oxygen consumption, proportion of fat used, and time required for recovery. As similar experiments were repeated, training tended to reduce these evidences of fatigue. It would have been instructive to have determined acetone bodies in urine in these experiments. Mills (18), following up earlier work of

Courtice & Douglas, has shown that one of two subjects after a ten-mile walk always had ketonuria; he attributed it to a low carbohydrate intake.

Peters & Zaeper (19) have concluded that the oxygen utilization from the blood in rest is greater than normal in the athlete; in this connection it has been shown that training guinea pigs to run increased the proportion of capillaries in cardiac and gastrocnemius muscles by 40 to 45 per cent (20). There was no change in number of capillaries in skeletal muscle not used in running. While dextrose ingestion reduced lactate formation in exercising normal and asthenic children, training was even more effective (21).

In a comparison of trained and untrained men on the bicycle ergometer, McNelly (22) found no difference in mechanical efficiency but in the former the R.Q. was lower, the oxygen debt smaller, and the percentage of oxygen removed from inspired air greater. Training is supposed by many to increase blood hemoglobin, but one week's training of dogs lowered the concentration of blood hemoglobin and the osmotic resistance of red cells (23). Simonson (24) has studied performance of runners both trained and untrained using a device for measuring speed and acceleration. Preliminary warming-up improved performance. Time in a one hundred-meter sprint was bettered by hypnosis.

Backman and associates (25) have described two patterns of variations in blood creatine and creatinine in man in relation to training. Šantavý (26) finds that the glutathione content of blood declines in exercise. This comes earliest in the untrained subject and latest in the best trained.

It is a common but not universal experience of the trained athlete to get his "second wind." It has been suggested (27) that the "dead point" of exercise just precedes the onset of second wind. At the dead point, oxygen consumption reaches a maximum and then levels off. Blood sugar is lowest and R.Q. highest; sweating begins with second wind. Training according to Simonelli & Ferri (28) doubles the ability to hold the breath, and a higher alveolar  $\text{CO}_2$  is tolerated.

Muscle soreness is a necessary concomitant of training. In soreness of the arm muscles experimentally produced (29), the arm undergoes an increase in volume that does not subside for 6 to 24 hours. Muscle soreness may persist longer; probably it does

not depend on imbibition of water or on metabolic products accumulated in work.

Rabbits trained on a treadmill show an increased sensitivity of tonic muscles to acetylcholine. Increased work capacity is associated with longer and stronger acetylcholine contractures (30).

The popular idea that fatigue depends on some elusive toxin has led to many fruitless searches for a cure. Within modest limits a recently developed drug, benzedrine sulphate, is such an elixir. Ingested in doses of 5 to 20 mg., it relieves fatigue arising from insufficient rest and sleep both in normal subjects and in certain neuroses associated with depression and anhedonia (31). There is no evidence that it influences fatigue arising from muscular exercise under ordinary conditions.

Kaunitz & Selzer (32) conclude from unsatisfactory experimental data that fatigue of muscles is associated with upset in oxygen utilization, alterations in cell permeability, and transfer of ions across altered membranes. Livers of fatigued rats seem to lose potassium and gain sodium.

Wickwire and associates (33) have found that the knee jerk gives a useful index to fatigue, whether physical or mental. As the day's work advances, a heavier blow must be struck to elicit a given response. Strenuous exercise has the same effect, particularly in the untrained man. They suggest that a standardized knee jerk test might give an index to physical fitness.

For a review of fatigue up to 1934, reference may be made to Simonson (34).

*Metabolic and respiratory adaptations.*—Considerable interest was aroused when Haggard & Greenberg (35) announced that mechanical efficiency is increased one-third or more after meals. They proposed that factory workers should have midforenoon and midafternoon snacks in order to increase their efficiency. Their thesis was tested in a factory manufacturing tennis shoes. The results were unmistakable: production increased when extra meals were offered. It does not necessarily follow that increased production depended on a change in R.Q.; in fact, the major premise of the argument is not in harmony with earlier studies nor with the report of Haldi and associates (36) that mechanical efficiency is not improved by taking food nor is it correlated with the height of the R.Q. before exercise. The R.Q., mechanical efficiency, and oxygen debt have been studied in health, inanition, and diabetes

by Alberts & Dietrich. (37) They conclude that fat is normally converted to carbohydrate and that the low mechanical efficiency in both inanition and diabetes depends on a loss of this function: fat is burned directly. Their experiments were based on ten-minute work periods and on this account may not be conclusive.

It has been shown by Carpenter & Lee (38) that in rest or work the metabolism of sucrose differs from that of galactose. This investigation illustrates an important principle: the resultant of two forces acting together is not easily calculated from the effects of these forces acting separately. "The metabolic effect of sugar ingestion accompanied by work did not represent a summation of the effect of the injection of the sugar . . . at rest and the effect of muscular work without sugar."

The R.Q. in obese women is usually below 0.8 in rest but above that value in work. The low resting values are attributed by Forbech (39) to transformation of fat to carbohydrate, but many will consider the evidence inconclusive.

Lehmann & Vennwald (40) have developed a method for measuring ventilation in rest or work with minimal handicap on the subject. A hot metal filament placed in a nostril changes in electrical resistance as a function of ventilation.

Schuster (41) has measured the effects of a gas mask on respiration in rest and work. In rest, ventilation decreased about one-third with no change in respiratory rate. In work, there was a decrease both in volume and rate when the mask was worn.

Studies of the circulation in the exercising animal can be made effectively with the Rein thermostromuhr. This has been perfected by Baldes and used at the Mayo Clinic to study the coronary circulation in dogs (42). The coronary blood flow increases rapidly as exercise is begun, soon reaching a plateau. With an increase in rate of work, a rapid adjustment of coronary flow is made. These changes do not run parallel to changes in blood pressure.

An apparatus has been developed by Beecher, Field & Krogh (43) for measuring venous pressure while walking. The pressure varied from 30 to 70 mm. of water, with a mean of 44 mm. in a typical experiment. The production and return of lymph can be explained on physical grounds from their data.

Recently Rein and his students (44, 45) have applied his continuous recording method for a study of oxygen consumption and carbon dioxide output in knee-bending with restricted blood flow

in man. Upon release of the constriction, there is a local hyperemia that depends on local chemical stimuli—chiefly oxygen lack. The recovery oxygen consumption remains above normal until after unoxidized end products are removed.

Kramer (46, 47) has developed a photoelectric method for estimating arterial blood flow that depends on an analysis of the rhythmic variations in oxygen saturation. The accuracy of Anrep's findings is confirmed in so far as blood flow in short tetanic contractions is concerned but in long-continued contractions he supports Rein.

Asmussen & Hansen (48) find that heavy static work carried on with the legs for one or three minutes produces a great increase in ventilation, oxygen consumption, and cardiac output after work. The results point to a restricted circulation to the legs during work—anaerobic processes must be employed.

When a group of muscles is apparently working at capacity, calling another group into action may increase the output of the first. This explains why vigorously swinging the arms at the end of a race may be advantageous (49).

Carrasco (50) has reported values for oxygen intake as high as five liters per minute. The method used—graphic recording on a closed system—should be checked by the open circuit procedure. Robinson, Edwards & Dill (51), by collection of expired air in a Douglas bag, measured the oxygen consumption of five leading distance runners. That of Lash reached 5.35 liters per minute—a value 21.4 times his basal oxygen consumption. A notable experimental study of heavy muscular work has been carried out by Christensen, Krogh, Lindhard, Bang, Bøje & Nielsen (52). Nielsen (53) has found that the ventilation can be increased in rest or moderate work during carbon dioxide breathing to 60 or 70 liters per minute, while in maximal work it can reach 100 to 120 liters per minute. The work of respiration increases as the vital capacity decreases in healthy young men. The work of respiration at rest requires about 0.5 cc. of oxygen per minute, while in maximal work it may reach nine per cent of the total metabolism.

The regulation of respiration has been discussed by Nielsen (54). More recently, Yandell Henderson (55) has surveyed the work of his own laboratory in this field. His researches have many practical applications in medicine and public health.

*Blood changes.*—After brief severe work there is an increase of

9 to 17 per cent in calcium and protein of plasma. The restraint of calcium within the blood stream means that it acts as an osmotic buffer under these conditions (56). According to Malorny and Netter (57), sodium moves from blood to muscle during work as lactate increases in blood. Even if true this cannot be looked upon as simple exchange, for presumably the sodium ion does not enter muscle cells and the lactate ion must be at least as concentrated in tissue fluid as in blood plasma. Keys (58) has shown that potassium and sodium of plasma behave very differently under the same circumstances. Sodium concentration in plasma is increased at the end of work by 2 to 10 per cent, but reaches normal in about fifteen minutes. Potassium concentration is 25 per cent increased at the end of work but drops below the rest level after ten to fifteen minutes and later exceeds it. The original plateau is regained after 1 to 1.5 hours. The cycle was observed in six subjects and does not depend on hemolysis or exchange with red cells.

The removal of lactate from blood is a logarithmic function of time, but the value of the function depends on the state of the organism. Newman, Dill, Edwards & Webster (59) find that moderate exercise increases the rate of removal; up to a certain level the greater the activity, the faster the removal rate. Presumably, increased circulation as well as greater use of lactic acid by muscles account for this phenomenon. Along the same line, Cowan & Solandt (60) find that when a base line of steady exercise is used as a standard, recovery from short severe exercise is complete within 20 to 45 minutes as compared with ninety minutes or more when a resting base line is used. Sacks & Sacks (61) conclude that changes in lactic acid in muscle and blood do not run hand in hand. They believe that lactic acid does not necessarily diffuse freely from muscle to blood and that this favors the maintenance of a steady state. This finding has not been confirmed by Newman (62) in a different type of experiment: after normal rats reach a steady state, lactate is equally distributed between muscle and blood.

Several years ago, Dennig and associates (63) in the Fatigue Laboratory studied the possibility of increasing the capacity for anaerobic work by previous administration of alkali. Dennig has followed this up (64) and announces that work capacity is significantly increased by ingesting sodium citrate (5 gm.), sodium bicarbonate (3 gm.), and potassium citrate (1.5 gm.), after meals. Along the same line, it has been reported (65) that increasing the



alkalinity of a horse's diet lowers the resting lactate and diminishes the lactate increase in work. While increase in lactic acid plays the major role in acid-base disturbance in exercise, other associated substances are known to appear in the blood. Pyruvic acid has been reported in the blood after severe exercise by Johnson & Edwards (66) and both methylglyoxal and pyruvic acid by Ivanenko and associates (67). Johnson & Edwards (68) have shown that the lactate and pyruvate recovery curves for blood and urine are similar in shape, although the concentration of pyruvate is less than one-tenth that of lactate. Pyruvic acid was isolated as the 2,4-dinitrophenylhydrazone.

*Renal function.*—There is no agreement regarding the effect of strenuous muscular activity on renal activity. Hellebrandt and associates (69) report that kidney function was depressed for 45 to 60 minutes after violent exercise. They attribute this to anhydremia, low arterial pressure, and disturbed balance of osmotic and hydrostatic pressure. Havard (70) finds a decreased chloride excretion and a normal urine volume, but according to Norris & Weiser (71) there may be not only a decrease in the rate of output of creatinine, uric acid, chlorides, and phosphates but also a diminished urine volume.

There may be increased excretion of creatine after exercise (72). The decrease in urea clearance in football and basketball players is not closely related quantitatively to the degree of albuminuria (73), but in both laboratory experiments and football play, the depression in clearance is roughly proportional to duration of strenuous activity (74). Covian & Rehberg (75) find a decrease in diuresis that runs parallel to the intensity of the work: greater reabsorption is associated with greater extrarenal loss of water. Filtration is diminished only in heavy work; it returns to normal early in recovery. Albuminuria of very severe work they attribute to anoxemia. Contrary to some of these observations, Krüger (76) finds no increase in creatinine after strenuous exercise and no change in urine volume or specific gravity. Such disagreement probably depends on variations in conditions of work and in the length of periods over which urine is collected.

*Physiology of industry.*—Physical standards in industrial health have been discussed by Munro (77). The Industrial Health Research Board of Great Britain carries on numerous practical studies, physiological and psychological in nature. These are re-

viewed in their annual report, available each fall (78). At the Kaiser Wilhelm Institute at Dortmund Basler (79) has made an extensive study of the physiology of the foot and the proper design of shoes and Müller (80) has determined the relation between the saddle height of the bicycle ergometer and the capacity for work. It was found that

$\log A = aN + b$ , where

$A$  = total work done in kg.-m.

$N$  = rate of work in kg.-m. per min.

The values of the constants  $a$  and  $b$  depend for a given subject on the saddle position. He measured work output at various rates of work on six men. Not only is there a great individual difference in capacity but individuals do not necessarily stand in the same order at different rates of work (81). Atzler, Lehmann & Szakáll (82) have studied the effects of caffeine on the intermediary metabolism in rest and work.

According to Myshkis & Myshkis (83), plasma water and carbon dioxide in boiler shop workers decreased throughout the day, while pH remained constant. In trained workers both alkaline reserve and lactic acid decreased during the day's work, but in untrained workers the latter increased. Chloride of plasma and usually of erythrocytes increased; these changes were opposite to those observed in strenuous exercise. Morphological changes in the blood observed in various industrial workers did not depend on duration or intensity of work (84).

Dill, Bock, Edwards & Kennedy (85) have discussed the question of industrial fatigue and described observations on steel workers. "The water and salt exchange in a group of steel workers is presented as an illustration of fatigue in which disturbance of physico-chemical equilibrium is an outstanding feature. It is shown that work under very rigorous conditions can be carried on provided the diet is suitable and the period of rest and sleep is adequate. In such activity the danger of breakdown is reduced by supplying cold drinking water containing 0.1 per cent salt."

The question regarding the adequacy of carbohydrate in the diet in men doing heavy work, raised by Haggard & Greenberg (35), can be answered by acetone determinations in urine where measurements of R.Q. are impractical. In our study of steel workers, no acetone was found under ordinary conditions. In this

connection Horres (86) has described a method for determining acetone in expired air. An increased excretion during strenuous exercise pointed to increased acetone formation as work went on.

Lahy (87) has studied fatigue and the organization of work in truck driving. Psychological tests, ergographic measurements, and pH tests of urine gave useful indices to fatigue. A trip of sixteen hours interrupted the normal regime of sleep, produced an alkaline change in urine, modified the outcome of psychological and ergographic tests. Individual differences were observed that depended on physiological make-up.

Atzler, before his untimely death in September, 1938, completed the first section of a review on work physiology in which special attention was paid to industrial physiology (88). The institute of which he was head, the *Kaiser Wilhelm Institute für Arbeitsphysiologie* in Dortmund and the journal that he edited, *Arbeitsphysiologie—Zeitschrift für die Physiologie des Menschen bei Arbeit und Sport*, occupy dominant positions in this area.

**Age**—The increasing average age of man is raising new problems in economics, in medicine, and in physiology. It is not out of place to refer here to some recent investigations in this field. Benedict and associates (89) have determined basal metabolism in rats more than one year of age. Males and females have the same values; in each sex it tends to increase with age in contrast with man. In middle age, male rats not previously exercised fail to adjust themselves to vigorous enforced work; they lose weight rapidly and die. Females are not similarly affected; they benefit by the exercise.

Benedict, Kung & Wilson (90) have studied the basal metabolism of Chinese in relation to age, sex, and activity. Women undergo a decrease in metabolism with advancing age that is twice as rapid as in Caucasian women; the latter do not lose vitality nor age so rapidly as the Chinese women. Those males engaged in active labor had a higher basal metabolism than sedentary workers. By every standard of comparison, Chinese have a lower basal metabolism than Caucasians.

Pre-adolescent boys show an increase in maximal oxygen intake in exercise with advancing years. The frequency of breathing in exercise is greater in boys than in men, and their respiratory dead space does not increase so much in exercise (91).

Robinson (92) has studied metabolic and respiratory adapta-

tions to work in subjects ranging in age from 6 to 76 years. Men in the seventh or eighth decades have lost about one-half of their capacity for transforming energy aerobically. The ability to supply blood to active tissues has decreased, and skeletal muscles are weaker. Nevertheless, when working within the limit of their ability, many men well advanced in years can carry on long-continued work with a lower heart rate and less evidence of fatigue than that exhibited by young men. The capacity for anaerobic work appears to be maximal in the early twenties and to be small in young boys and old men. Robinson found that the highest lactate levels are reached in the twenties, while there is only a slight lactate increase in the hardest work attempted by young boys or old men. It is reasonable to conclude from these observations that occupations with the emphasis on speed and strength are best suited to young men. Older men are best fitted for jobs requiring skill, co-ordination, and endurance.

#### EFFECTS OF HIGH TEMPERATURES

Bazett's laboratory is a center for research on the effects of high temperatures. Burton & Bazett (93) have employed an insulated bathtub as a useful tool in measuring heat loss from an immersed surface. Physical regulation balances heat loss so long as the temperature gradient from the interior of the body to the surface does not exceed four degrees C. It is achieved by vasomotor alteration in the effective thermal conductivity, defined as the ratio of heat flow per unit area to the temperature gradient, which may change by a factor of about six. Subjects are more susceptible to dehydration in hot baths in winter than in summer (94). As circulatory failure approaches, very large arterio-venous differences are found—an indication of low venous saturation. Bazett now reports (95) that blood volumes of healthy men in Philadelphia are 15 to 40 per cent higher in summer than in winter. Furthermore, men exposed for a few days to high temperature in the midwinter experience an increase in blood volume, although not so striking as in summer. These findings have been discussed by the author elsewhere (96). It will be interesting to see how fully they are borne out by further experiments in other hot climates.

Winslow and associates have measured the thermal effects of various environments on two male subjects at rest. Under the experimental conditions, sweating began at a skin temperature of

34.5°. Between 25.5° and 31° evaporative heat loss is minimal; it does not change much with a further decline in temperature. As the operative temperature drops from 31° to 20° skin temperature drops from 34.5° to 29°. Vasoconstriction begins over the whole body surface at once; it effects a reduction of one-half in conductivity and presumably in cutaneous blood flow between skin temperatures of 35° and 32°. They express (97) the net effect of different combinations of air and radiant wall temperatures in determining heat loss from the body in terms of an operative temperature. Thermal adjustment of the body to a new environment is favored when wall and air temperatures are widely separated. Gagge (98) has defined the variable  $w\mu$  as the effective moisture surface present on the skin surface of the body.  $w$  is unity when  $w\mu$  is maximal and when  $\mu$  is 28.5. The authors believe that when all sweat is not being evaporated the evaluation of this function is useful. It depends on measurement of body surface, metabolism, heat storage, radiation, and convection exchanges, skin temperature, relative humidity, and air temperature. Three men subjected to a wide range of environmental conditions responded in a similar fashion despite a range in body weight from 51 to 105 kg. (99). The discharge of sweat closely followed the evaporative demands of the environment. In our experience the responses of healthy men subjected to stress are as notable for their differences as for their uniformities. Some sweat too much, others not enough. Some put out dilute sweat and become thirsty; some put out concentrated sweat and undergo a decrease in volume of extracellular fluid without much thirst. It is hoped that the elegant methods of analysis of environmental effects developed in Winslow's laboratory will be extended to individuals that differ in their methods of adaptation.

The long series of observations made by DuBois on heat dissipation and temperature regulation have been summarized recently (100). Since then, Hardy, Milhorat & DuBois (101) have carried out measurements of the amount of heat lost by various avenues in relation to air movement and clothing. Between 27.4° and 31° C. an electric fan increased convection, but there was no change in basal metabolism or rectal temperature. At 34° the fan had no effect on heat lost by convection. A comparison of clothed with unclothed man showed that the surface of the clothing was 1 to 2° cooler than the skin but the vaporization was about the same.

Adolph, Dill, Hall, and Edwards carried out experiments on water balance and temperature regulation in the desert in the summer of 1937 (102, 103, 104). The modified water balance involved an average eight-fold increase in evaporation, while urine volume remained constant or decreased. The specific gravity of urine increased. The maximal observed rate of evaporation was 1.7 liters per hour and the minimal rate of urine formation, observed during work, was 10 cc. per hour. Water intake accurately balanced loss except in exercise, when it tended to lag behind. The daily turnover was ten to thirty times the daily variation in body weight.

For a given rate of sweat formation, its concentration decreases during adaptation to heat. As sweating becomes more profuse, its inorganic constituents increase in concentration, while nitrogen decreases. The susceptibility of some individuals to heat cramps no doubt depends in part on the inability of their sweat glands to reduce salt losses to a low level. Adolph compared rates of heat production, of gain by radiation from the sun, of loss by evaporation, and of heat accumulation. While resting in the sun, radiation may add heat to the body faster than it is produced chemically. In exercise, along with high rates of heat production, radiation and convection may add so much heat that the loss by evaporation alone may exceed heat production. Recovery from exercise required up to 1.5 hours for dissipation of accumulated heat. During exercise, pulse and systolic arterial pressure increased with rate of heat loss, indicating an increased role of the circulation. Changes with successive rates of exposure occurred in rates of evaporation, heat accumulation, and pulse. Individual differences in effectiveness of temperature regulation were noted; these depended in part on variable rates of sweat production. Mezincescu (105) has found that the salt concentration in sweat decreases during a period of acclimatization in Boston in midwinter. There is also a dependence of sweat chloride on the level of salt reserves in the body. The chloride content in 53 samples of sweat from seven men ranged from 0.1 to 0.5 per cent, averaging 0.3. About one-half the nitrogen was in urea and one-fifth in ammonia. The pH ranged from 6.7 to 8.4 but usually varied between 7.1 and 7.5.

Various investigations of sweating by the Haldane school have been summarized by Whitehouse (106). He believes that osmotic water loss through the skin varies with atmospheric conditions

and muscular activity—the rate of loss depends on peripheral circulation. During continuous sweating the acidity of the protective epidermis is reduced, lowering its bactericidal efficacy. We were not able to confirm the latter conclusion at Boulder City. In some cases sweat became more alkaline as sweating continued but in others it became more acid.

McCance (107) produced salt deficiency experimentally and noted reductions of 28 to 38 per cent in volume of extracellular fluid and a variable fall in NaCl concentration in sweat on successive days—a change to which adaptation did not contribute. Some individuals, particularly women, were resistant to the regime—they soon dropped to a very low level of salt concentration in the sweat. Gastric juice was little changed, but the sodium content of saliva and of cerebrospinal fluid was reduced. The maintenance of normal hydration favors temperature regulation and increases rate of sweating as well as the efficiency of circulatory functions (108). Lehmann & Szakáll have found that in a person untrained to work in a hot environment some days' work on a low chloride diet with excessive sweating caused severe symptoms that were relieved by administering salt solution (109). The pulse rate was raised both in rest and work, and vasodilatation was observed. For work in heat, gradual adaptation is necessary and the water intake during work should not exceed one-half the volume of sweat. They believe that drinking saline hinders adaptation but relieves disturbances in the untrained person. Not only heat cramps but also heat exhaustion may involve, at least in part, salt loss and diminished volume of extracellular fluid. The phenomenon of adaptation may depend on increased blood volume, as observed by Bazett (95).

Heat exhaustion was experimentally induced by Weiner (110) in Bantu workmen of the Witwaterstrand mines. The collapse and associated circulatory findings could be explained by peripheral vasodilatation. Differentiation from heat stroke and heat cramps was made. In this connection a case of heat exhaustion has been reported that resulted when a workman left an ice house at  $-2^{\circ}$  and came into the open at  $20^{\circ}$  (111). In 44 cases of heat stroke it has been proved that a high body temperature is the chief cause of the symptoms and absence of sweating the chief precipitating factor (112).

Ascorbic acid was proved to be present in sweat to the extent



of 0.55 to 0.64 mg. per 100 cc. by Cornbleet and associates (113) in 1936. The following year, Bernstein (114) showed that because of this loss miners in the Witwaterstrand develop scurvy on what would be otherwise an adequate diet.

According to Niederhäusern (115), the oxygen consumption after a period of standard work is about one-fifth greater in the tropics at 26 to 30° than at Bologna at 16 to 18°. If this means a change in mechanical efficiency, it is contrary to accepted ideas and to such recent observations as those of Liberson (116). The application of physiology to industrial situations in which high temperatures and humidities are experienced has been discussed by Crowden (117).

In dogs the stomach empties more rapidly at low, less rapidly at high temperatures than normally. It is suggested that this may have some bearing on the effects of high temperatures on appetite (118).

Many physiologists think that the panting of dogs is so shallow that the properties of the blood are little altered. Hemingway (119) has devised a method for measuring ventilation in dogs panting normally: ventilation may be increased ten times while tidal air is reduced nearly one-half. A lowered tidal volume, "according to theory is desirable to prevent loss of carbon dioxide by hyper-ventilation." Whatever theory one adopts, it is a fact that dogs subjected to high temperature respond by an effective increase in alveolar ventilation. They tolerate alkalosis when heat must be dissipated rapidly, as was proved years ago by Rice & Steinhaus (120).

Laurens' monograph on the effects of radiant energy is well known to workers in applied physiology. Recently he and Foster (121) have compared the responses of eleven males, including one negro and one Japanese, to various wave lengths. Contrary to the general impression, there was no great difference between negro and white. Temperature responses indicate that no wave lengths can be considered penetrating from  $4\mu$  to the visible range in the sense that little energy penetrates to a considerable depth. Natural pigment offers little protection against short infra-red and no protection against long infra-red. The skin acts as a black body towards long infra-red but not towards short infra-red and visible radiation.

Leonard Hill (122) has called attention to the effect of radiat-

ing the skin on narrowing of the airways of the nose and lungs. "When people go out of doors to get a 'breather' they are instinctively using the stimulus of cold to widen reflexly their air tubes. In open-air treatment the same effect is obtained; so, too, when sleeping in a room with open windows." It is suggested that the capacity to breathe through orifices of varying size may be an index to physical fitness. Lehmann (123) has determined that differences observed by Hill between the effects of long and short waves depend on the depth to which they penetrate; the stimulus depends on the temperature gradient between the surface and the deeper layers of the skin. Similar dilating and constricting reflexes, dependent on temperature gradients in the skin, regulate capillary flow in the nose as well as in the epidermis.

#### LOW AND HIGH OXYGEN

*Low oxygen pressure.*—In a modified rebreather test for aviators the volume is increased to 200 liters and the initial percentage of oxygen adjusted to 13. This saves time early in the test and prolongs the critical period. Six subjects with first-class rating reached an alveolar ventilation of 14.4 liters per minute, while six with a poor rating reached only 7.6. For a first-class rating men should reach without serious anoxemia a  $pO_2$  of 60 mm. on the rebreather or withstand 360 mm. in the pressure chamber for ten minutes (124).

Many of the tools of physiology can be applied only with difficulty in low oxygen pressures. Christensen & Nielsen (125) have mastered the problem of measuring cardiac output by the foreign gas method. Cardiac output tends to be higher both in rest and work. Matthes & Malikiosis (126) have succeeded in registering oxygen saturation of arterial blood continuously in low oxygen. A complete equilibrium between capillaries and alveoli existed. Using the Fick principle, they, too, find an increased cardiac output. Benzinger (127) has been able to record ventilation, carbon dioxide output, and oxygen consumption continuously while the subject undergoes changes in atmospheric pressure. He finds a considerable variability in effectiveness of alveolar ventilation but a favorable influence on it of muscular work. Various other investigations along this line have been reviewed recently (128).

The pneumogram has been used by Thompson (unpublished data) with some success in classifying airmen with regard to adap-

tability for flying, and it is therefore of interest to note that Tavel (129) has devised an apparatus that will give a satisfactory pneumogram in a low-pressure chamber. Questions of blood pressure and respiration in low oxygen are dealt with in other papers (130, 131, 132, 133).

Respiratory regulation in low oxygen is greatly improved by the presence of carbon dioxide (134, 135). The advantage is so great that some air lines have considered releasing carbon dioxide in their cabins at high altitudes; since it can be carried in cylinders as a liquid, the tare weight is much less than with oxygen. The practicability of using carbon dioxide is doubted by Jongbloed & Wildschut (136); they prefer oxygen.

Adaptation to low oxygen probably depends in part on circulatory adjustments. Schwarz (137) frequently observed collapse among the youngest of the flight apprentices he examined in low oxygen; he attributed this to unstable vasomotor systems. Similarly (138), mice one year old withstood low oxygen better than very young or very old mice. Herbst & Manigold (139) found little evidence of circulatory adaptation until the pressure dropped below 477 mm. Hg.; at 350 mm. signs of breakdown appeared and at 250 mm., syncope.

It has been suggested that oxygen lack may modify capillary permeability, but McMichael & Morris (140) have been unable to confirm this in man. Intracranial pressure increases in carbon monoxide poisoning, and Michelsen & Thompson (141) have discussed two cases of acute oxygen lack with characteristic signs of increased intracranial pressure.

Adaptation of mice as influenced by repeated exposure to carbon monoxide has been studied by Lehmann (142) and of rabbits, by suddenly reducing the pressure to extreme limits, by Armstrong & Heim (143).

The adaptive changes that occur in the blood in long-continued exposure to low oxygen are well known, but in acute oxygen lack they are less well defined. Changes in carbon dioxide, oxygen, and pH in a dog under anaesthesia can be shown continuously by a graphic method (144). Blood sugar showed an average decline of 14 mg. per cent in eighteen men exposed for one hour to 402 mm. Hg. (145). When exposure was continued to the point of altitude sickness, blood sugar usually increased (146). Serum calcium in acute anoxemia declined on the average about 0.5 mg. per cent

in twenty experiments (147). A study at an altitude of 6,000 feet has been reported by Apperly (148). The hematocrit, after an early increase, returned to normal. Values of pH were estimated from chloride distribution between cells and plasma, and alkaline reserve by equilibrating blood with alveolar air at room temperature. Where changes are small, as must necessarily be the case at 6,000 feet, more precise methods should be used.

When dogs breathe air at  $-20^{\circ}\text{C}.$ , their arterial blood is cooled  $0.4^{\circ}$ . In low oxygen this has a slight but unimportant advantage in increasing the affinity of blood for oxygen in the lungs (149).

Gellhorn (150) has demonstrated that mice cannot survive a low  $\text{pO}_2$  at high temperatures as long as at room temperature. At room temperature and a barometric pressure of 295 mm. Hg., the body temperature fell  $3^{\circ}\text{C}.$  in ten minutes, but the mice survived. With 3 per cent of  $\text{CO}_2$  added, rectal temperature fell even more in the same length of time. At  $37^{\circ}$ , nine out of ten mice died after nine minutes' exposure to a barometric pressure of 295 mm. It would have been interesting to have continued the exposure to low temperatures longer; obviously the mice were in a precarious state with an unchecked fall in rectal temperature taking place.

Ammonium chloride ingestion has been proposed as improving tolerance to low oxygen, but Barron and associates (151) found no evidence for its practical advantage. They suggest that the oxidative system within the cell may hold the key to variability in response to the same arterial oxygen concentration—a suggestion given experimental support by Hurtado and associates (152), who demonstrated that in dogs acclimatized to high altitudes the concentration of myoglobin in tissues is increased. The role of glutathione of cells in oxygen lack has been discussed by Gabbe (153).

There is not much to be said about the subject of diet and oxygen lack except that this is a question of serious concern to the commissary departments of air transport organizations. There is a disturbed acid-base balance at high altitudes (154), but it is doubted whether the remedy proposed (155), an alkaline diet, is adequate. Curiously enough, it has been reported that rats are rendered resistant to low oxygen pressures by a diet of carrots (156).

Christensen & Nielsen (157) have found that the strength of skeletal muscles as measured on Mosso's finger ergograph or on Hill's wheel is the same at 760, 440, and 390 mm. Hg. Hartmann

(158) reported some decrease in the unacclimatized subject at about 440 mm., but no change in the adapted subject until less than 350 mm. is reached. The functional resistance of muscle to oxygen lack exceeds that of the central nervous system (159).

The resting metabolism is usually normal in low oxygen pressures, and the oxygen intake in work is normal or below normal, but recovery may be delayed—the oxygen debt is greater. The failure to keep pace with the demand for oxygen may depend in part on temporary damage to the respiratory center from acidosis and hypocapnia (160). Heymann (161) has measured the oxygen consumption in fixed work in 14 and 21 per cent oxygen. By this method those best fitted for activity at low  $pO_2$  can be selected; acclimatization complicates matters, for some accomplish this more successfully than others. At the end of a seven-day fast, the capacity for sustained work and the stability in low oxygen are reduced, but muscle strength is unchanged (162).

The effects of trans-Pacific flights at 8,000 to 12,000 feet on airmen and passengers have been studied by McFarland & Edwards (163). There was a tendency towards polyuria, particularly early in the flight, in those who had the greatest responsibility. There was evidence of acclimatization, indicating that during ordinary flight conditions a high degree of mental and physical efficiency is maintained. Passengers did not manifest as much adaptation as pilots but showed no objective signs of fatigue or physical distress. In the same connection, Mangiacapra (164) has found that the urinary pH may increase one unit in a short flight by inexperienced airmen and in some experienced pilots. During prolonged flights the urinary pH decreases from 0.2 to 0.8 units, the extent depending on duration of flight and characteristics of the individual. Medical viewpoints regarding fatigue in relation to flying have been expressed by Porter (165), Miller (166), and Fenwick (167).

Among recent reviews of practical interest are those by Klemperer (168), Armstrong (169), and Heim (170). These deal with medical and physiological problems of high altitude flight. The various reports of the International High Altitude Expedition to Chile in 1935 (151; 171–187) have been summarized by Dill (96), Keys (188), and McFarland & Dill (189).

*High oxygen pressure.*—Rats exposed to six atmospheres of oxygen for thirty minutes and decompressed slowly rarely survive

at 31 to 35° and rarely die at 21 to 27° (190). It also appears that thyroidectomy protects against oxygen poisoning. Campbell proposes that the usual oxidative processes of nerve centers are accelerated and that the thyroid gland plays an important part in this toxic action (190).

It is many years since Sayers, Yant & Hildebrand pointed out the theoretical advantages of helium-oxygen mixtures in deep-sea diving. Behnke and associates (191) found that in the treatment of compressed-air illness in dogs there is considerable advantage in the substitution of helium for nitrogen. Recently End (192) has carried out experiments on man, using the new Craig-Nohl diving equipment. The diver carries small cylinders of helium and oxygen with valves so arranged that oxygen is admitted at a suitable rate and at the proper partial pressure, while carbon dioxide is absorbed. Two subjects were decompressed in one twenty-third the time ordinarily required. Craig, one of the subjects in this experiment, has given a popular account of his experiences (193).

Breathing 45 to 47 per cent oxygen increases the capacity for work provided there is time for circulatory adjustments. The advantage is not due merely to extra dissolved oxygen carried to skeletal muscles; evidently the circulation is improved. Ventilation and R.Q. are less; apparently more fat is used (194).

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FATIGUE LABORATORY  
MORGAN HALL, HARVARD UNIVERSITY  
BOSTON, MASSACHUSETTS

## ENDOCRINE GLANDS: GONADS, PITUITARY, AND ADRENALS\*

BY HERBERT M. EVANS

*Institute of Experimental Biology  
University of California, Berkeley, California*

### SEX HORMONES

The recent brilliant period of successful isolation and chemical characterization of the male and female sex hormones has now been succeeded by an epoch of confusion. This is not to say that the doctrine of the establishment and accentuation of the male and female accessory reproductive systems and secondary sexual characteristics by the male or female sex hormones respectively has been shattered; this has been increasingly established and is beyond cavil. The present epoch has been characterized as one of confusion because of the demonstration of the close chemical interrelationships of all the sex hormones (including progesterone) and the possibility of conversion of one to the other (it will be remembered that the urine of the stallion is one of the most potent sources of estrone, whereas it is not found in the urine of the gelding), the isolation of male hormone from actual ovarian tissue, the fact that all males secrete estrogens and females androgens. All these matters are now well established facts. It would appear that maleness or femaleness can not be looked upon as implying the presence of one hormone and the absence of the other, but that differences in the absolute and especially relative amounts of these two kinds of substances may be expected to characterize each sex and, though much has been learned it is only fair to state that these differences are still incompletely known. Three groups of expert workers resident in London, in Amsterdam and in Chicago, respectively, have been intensively

\* Fortunately, during the last year there have appeared six important summaries (1, 2, 3, 4, 5, 6) of our knowledge of the whole or part of this field. Consideration of the pancreas, thyroid, parathyroid, thymus and pineal has regretfully been omitted from this review; even in the domain of the three important endocrines chosen, during the last year (1938) over two thousand articles based on actual research have been published. A few hundred of these have arbitrarily been culled for this report but the reviewer fully realizes that many papers of significance have been omitted and some of lesser import included. The need for an "annual review of endocrinology" is upon us.

concerned, for example, with the estimation of urinary androgens under well controlled, as well as very diverse, conditions, and a member of one of these groups has declared that the results to date "emphasize the lack of a simple relation not merely between administered and excreted hormone but also between a physiological state and hormone in the urine. Any superficial theory which connects androgen excretion with male sexual function breaks down completely when it is shown that men can not be distinguished from women, or normal men from eunuchs in respect of urinary androgen." The intermediary metabolism of the hormones and even of administered purified hormones is far from clear. It has long been known that only an insignificant proportion of administered estrone can be recovered; and an administration of testosterone may lead to no recovery whatever. A decade ago Marrian (1929) detected an inert conjugated estrogen—a glucuronate—in the urine, and the brilliant demonstration by Venning & Browne (1936) that the glucuronate of pregnandiol is a breakdown product of progesterone along with the discovery of Pincus and-Zahl (7) that coincident progesterone assists the conversion of estrone to estriol represent the beginning of light in a dark realm—the metabolism of the sex hormones. The Montreal work has now been extended so that we have not only quantitative data on progesterone secretion in pregnancy but also in the normal menstrual cycle. It is not too much to hope that the conditions under which a known dosage of progesterone is utilized by the body or excreted will rapidly become known. The conception of the development of the male and female genital systems out of a common fundament, founded by the classic work of Johannes Müller, has been brought to a brilliant and convincing test by the creation of intersexuality in mammals through the administration of high doses of either sex hormone to pregnant animals. Some time ago Willier (1935) and subsequently Dantchakoff began to report the effects of such experiments and Greene, Burrill & Ivy (8, 9, 10, 11, 12) have recently published further studies of the striking masculinization of genetic female rats and the feminization of males produced in this way. Unquestionably human hermaphroditic changes could be produced, if they are not caused, by similarly acting factors, for Van Wagenen & Hamilton (unpublished) have now secured similar changes by sex hormone treatment of the pregnant rhesus monkey. Chemical



syntheses have achieved notable triumphs in the production of new estrogenic substances, some of them with a potency exceeding that of estrone. It has been known for some time that these need not be related chemically to the carbon skeleton of the naturally occurring estrogens. So also a new pregnen derivative has been shown to be more effective than progesterone in the specific effect of the latter. A few years ago Kaufmann succeeded in bringing about the complete development of the genital system, after its atrophy, in a doubly ovariectomized woman and to carry this to the provocation of menstruation. The experience was hailed as a theoretic triumph although enormous dosage with prohibitively expensive substances had been employed. Clauberg (13) and more recently Guldberg (14) have given the required proof of a similar accomplishment with some of the new synthetic substances. Among the most interesting achievements of the year have been discoveries as to the effects of various modes of administration of the hormones. Reference is made to the enormously increased effects which may be secured by the implantation of hormone substances in dry form, either as crystals or compressed tablets. The experiments show convincingly that exceedingly minute quantities of hormone are required for maximal effects providing a continuous rate of absorption is contrived in this way. But of even greater practical importance are the startling but well authenticated findings as to the efficacy of percutaneous administration. The actual beginning of this experience dates back to a few years ago when androgens were directly applied to the comb of the capon or chick. Zondek (15) then showed the remarkable efficiency of estrogens painted as creams or in solvents such as benzol on the shaved back skin. Moore, Lamar & Beck (16) have described the maintenance of the accessory organs of reproduction in castrate males by the application of ointments to the skin and the clinical literature, as might be expected, has already carried an extension of this to the human field. Finally, there has come convincing proof of the effectiveness of no inconsiderable number of these substances *per os*.

*Estrogen tests and assay methods.*—A number of new methods for the recognition or assay of estrogens has been published during the year. Astwood (17), by a careful study of the time relations between the growth and water exchange of the uterus following the administration of estrogen, states that the weight increase in

the uterus of the twenty-one day old rat has a direct logarithmic relationship to the dose of estrogen. Szarka & Kurtz (18) in a comparison of uterine and vaginal changes due to estrone, state that the distended estrous uterus occurs twenty-four hours sooner than cornified cells appear in the vagina. It can be made to appear at the fourth-fourth to fiftieth hour after the first injection, and he recommends its detection by laparotomy. The uterine unit is at least five times the vaginal unit. Collins *et al.* (19) state that experimentally induced heat in the guinea pig can be put on a rigid quantitative basis with reproducibility of results. The enlargement of the nipple of male guinea pigs has been proposed as a specific and a quantitative test for the female sex hormone. But Bottomley & Folley (20) show that certain unsaturated androgens, with the exception of androstenedione, exhibit definite teat growth promoting activity, transandrostenediol being the most active. At least three significant publications on the colorimetric estimation of estrogens have been produced; those of Venning *et al.* (21), Kober (a modification of his original method) (22), and Bierry & Gouzon (23). Marrian (24) has published a description of the mechanism of the Kober color reaction, carried out in conjunction with Darrach, in the course of which it is stated that it has been possible to "standardize the conditions for the color reaction of estriol with perchloric acid, so that it is now possible to determine quantitatively the amount of estriol in an estriol-estrone mixture by a direct process."

*Estrogens: biological action.*—Corner (25) has 'given an excellent review of the sites of formation of the estrogenic substances. His analysis of existing data leads him to the conception that they are usually produced by the theca interna of the ovarian follicles regardless of size whereas, in pregnancy, estrogens are "almost certainly" produced by the placenta. Edgar Allen (26) has supplied an admirable summary of the reactions of the genital tissues to estrogens. Soule (27) has shown that estrogenic hormones are present in equal concentrations in both maternal and fetal circulations. The latter fact is in harmony with certain endocrine abnormalities of the new born, for example, enlarged breasts and bloody vaginal discharge (cf. Neumann, and Philipp). The deleterious effect of high and long-continued dosage with estrogens has been further explored. Castillo & Sanmartino (28, 29) describe in rats subnormality of the thyroid, testes, ovaries and male

accessories but the uterus and vagina were hypertrophied. The adrenal cortex was increased as is the case in castration (although the glomerulosa was thin) and the hypophyses were enormous, in some cases reaching 175 mg. instead of 10 mg. Barks & Overholser (30) have shown that the myometrium is specifically affected by estrone injections, the circularis more than the longitudinalis, both hyperplasia and hypertrophy of the muscle cells occurring. Bell & Robson (31) have again shown the great increase in sensitivity to oxytocin and increase in the spontaneous activity of the uterine muscle of the mouse when spayed animals are treated with estrone, and were unable to modify the superactivity of such estrone treated uteri by progesterone or testosterone. Grumbrecht & Loeser (32) state that injections of large amounts of estradiol monobenzoate diminish the basal metabolic rate in normal female rats. They attribute this to diminished production of the thyrotropic hormone on the part of the pituitary. Shapiro (33, 34) has produced a decrease in urinary output in patients to whom large doses of estradiol benzoate were administered. His cases include two instances of diabetes insipidus (*vide infra*). Dessau (35) has continued studies with the chronic administration of estrone to guinea pigs, and, though there is no evidence of carcinogenic action, various forms of hyperplasia and epithelial metaplasia occur. Nelson (36) also has shown that cystic glandular hyperplasia of the endometrium is regularly produced by such treatments and a considerable number of subperitoneal fibromyomatous growths were found. Finally, Gardner *et al.* (37) have induced true squamous cell carcinoma of the cervix in the mouse by estrin. Balo & Purjesz (38) have further explored the peculiar sensitiveness of dogs to massive estrin doses. Such dogs die with decreased erythrocytes and platelets, although the leukocytes are increased and the bone marrow resembles that of myeloid leukemia. They found, as have others, that the cylindrical epithelium of the prostate is transformed to one of the stratified squamous type. Byrom (39) has shown that the sensitiveness of the rat to toxic doses of vasopressin is greatly enhanced by preliminary estrogen administration. Burdick & Whitney (40) have shown an acceleration of the rate of passage of fertilized ova through the uterine tubes of rabbits when 100 to 300 rat units of Progynon-B are given immediately following ovulation. A similar acceleration occurs in the treatment of mice but in the case of both animal forms, the accel-

erated ova degenerate promptly on reaching the fluid of the uterus so that pregnancy can not occur. Ball (41) has shown that on chronic therapy with a low level of estrin, female rats show only a constant low level of receptivity unlike the rhythmic peaks of normal animals. The treatment is therefore a mild sex depressant. Meyer & Wertz (42) have shown that although after thyroid administration the estrin threshold is higher, this is never increased over fifty percent, corresponding therefore nicely with the general increase in metabolism, in which connection in addition to the possible increased tissue need for estrin, it is conceivable that the increased metabolism of estrin itself must be considered.

*Estrogens, menstruation, progesterone.*—It is well established that only an insignificant portion of administered estrogen can be quantitatively recovered in the urine—three to twelve percent—the liver probably playing a major rôle in its destruction. Doisy's discovery (1935) of estradiol as the primary or true ovarian hormone has led to the conception of its conversion into estrone and estriol, the two urinary substances. Important light on estrin metabolism was shed by Pincus & Zahl (7) and Pincus (43) in their discovery that if estrone is administered to a pseudopregnant rabbit, or to an ovariectomized one treated with progesterone, estriol appears in the urine. Experiments carried out with hysterectomized animals showed that the uterus was necessary for the hydration of estrone to estriol. Smith, Smith & Pincus (44) and Smith & Smith (45) have published curves for total urinary estrogens, estrone and estriol throughout a menstrual cycle and in pregnancy. More estrone than estriol is excreted during the luteal phase than during menstruation itself or during the follicular phase. A rise in estrone accompanies the onset of menstruation. In pregnancy the estriol is always higher than the estrone after the second missed period, but at the onset of labor there is a drop in estriol and a rise in estrone. They state that the "results in the human are in keeping with the assumption that the ovarian product estradiol is converted into estrone, and that the degree of conversion of estrone into estriol depends on progesterone." They suggest that both menstruation and labor may be occasioned by progesterone deficiency which, no longer converting estrone to estriol, permits estrone to accumulate and precipitate these effects. It had at first seemed difficult to accord progesterone any essential rôle in menstruation because of the occurrence of menstruation in

women and monkeys in anovulatory cycles. Zuckerman (46, 47) has conducted an elaborate quantitative study of the reactions of spayed monkeys to estrone injections. With Long, he has made a new suggestion as to the cause of the menstrual cycle. Spayed monkeys injected regularly with a constant dose of estrone nevertheless exhibit cyclic bleeding and he feels that there is a cyclic fluctuation in the threshold of the uterus to estrogenic stimulation. The cycles therefore imitate estrin deprivation. He has suggested an anterior pituitary cycle causing an adrenocortical cycle which in turn causes cyclic hydration and dehydration of the uterus, the latter occasioning cyclic variation in the response to a constant level of estrogen. As will be disclosed, progesterone, however, is almost certainly involved in the menstrual mechanism whether endometrial evidence indicates an "anovulatory" cycle or not. In this connection it is to be noted that urinary pregnandiol glucuronidate has occasionally been found associated with endometria which are not in the secretory or so-called luteal phase. We may have to entertain the concept of the normal secretion of progesterone-like material by the adrenal cortex. Smith & Engle (48) have shown that in monkeys which have been both spayed and hypophysectomized the period elapsing between the cessation of estrin injections and the beginning of uterine bleeding was from one to somewhat over two weeks; but when pretreatment with estrin followed by progesterone was stopped, the bleeding occurred within forty-eight and a similar forty-eight hour bleeding occurred when combined estrin and progesterone treatment was stopped. Hisaw & Greep (49) showed that both estrone and progesterone in proper doses have power to inhibit uterine bleeding. Estradiol and progesterone given together will, however, inhibit bleeding at dose levels that are not effective when either is given alone. If progesterone is withdrawn after it has been simultaneously injected with estradiol, bleeding ensues. Corner (50) has also shown that when estrone and progesterone are administered at the same time, in suitable dosage, to castrated monkeys, discontinuance of the progesterone is followed by menstruation-like uterine bleeding in spite of the continuance of estrone treatment at a level more than sufficient to prevent estrin deprivation bleeding. A milestone was created by the success of Corner & Allen in achieving the continuance of pregnancy to term in the rabbit by the injection of crude progestin immediately following double ovariectomy in the

course of a pregnancy. With the purification of progestin this result was not secured by a number of investigators employing crystalline progesterone and the suggestion was made that concomitant estrin, known to be present for example in human corpora lutea, was required along with progesterone. Klein (51) has reported the inefficacy of progesterone in the hamster and stated that an estrin-progesterone mixture preserved the pregnancy after double ovariectomy in this species. Pincus & Werthessen (52) have, however, recently published an account of two instances in which after ovariectomy in the rabbit progesterone alone was successful in inducing implantation and protecting the pregnancy throughout its course, and a similar report has been made by Courier & Kehl (53) although the last mentioned investigators have shown that the demanded dose of progesterone may be greatly lowered by an exceedingly small amount of concurrent estrone. The necessary concurrence of estrin with progesterone in the formation of an histologically typical pregravid endometrium was indicated by the studies of Hisaw, Greep & Fevold (1937) and again by those of Engle & Smith (54) and make it extremely probable that the concurrence of a certain amount of estrin is involved in implantation. Such considerations impel one to inquire as to whether the success of Pincus and of Courier may not have involved the presence of a small amount of autogenous estrogen. Allen & Heckel (55) have re-studied this situation and leave us in no doubt regarding the efficacy of progesterone alone in maintaining pregnancy in the rabbit if castration is done as late as the eleventh day. The antagonism as well as the synergism of estrogens and progesterone has been irrefutably established. It is well known that progesterone inhibits estrus and ovulation, good quantitative work (in spayed animals) indicating that considerably over ten times as much progesterone is needed to inhibit a definite dosage with estrone. Unlike the estrogens and the androgens, progesterone, which has been called the only true female sex hormone (56) does certainly show a remarkable specificity, for many modifications in its chemical structures have been made by Butenandt and others and have been found devoid of action; even two isomers are inactive.<sup>1</sup> The history of the discovery of sex hormones of greater potency than those occurring naturally may in the

<sup>1</sup> It may parenthetically be remarked that almost two years ago the progesterone-like action of certain levels of testosterone was established.



future be repeated in this sphere for Inhoffen & Hohlweg (57) have reported pregnen-in-one-3-ol-17 is a very much more potent agent than progesterone for the latter's specific effect, 4 mg. *per os* accomplishing more than 60 mg. of progesterone parenterally. Van Dyke & Li (58) have recently studied the functional life of corpora lutea in the cat, produced by gonadotropic treatment, finding it to be in the neighborhood of twenty days. It will be remembered that the cat, which resembles the rabbit in that ovulation depends on coitus, is strikingly different from the rabbit in various endocrine thresholds, Rowlands & McPhail (59) having shown that seventy times the rabbit dose of estrone is required to sensitize the cat's uterus to progesterone. Van Dyke & Li show a similar phenomenon as regards gonadotropic hormone, the rabbit ovulating dose being a fraction of a percent of the cat ovulating dose. Van Dyke's evidence of the secretion of progesterone was derived from the response of the isolated cat uterus to adrenalin. This so-called pregnancy reversal, *i.e.*, contraction in response to sympathetic stimulation, is regarded by him as a specific progesterone test more sensitive than the morphological changes. They call attention to the fact that in the cat there is no correlation between uterine sensitivity to posterior lobe extracts and the function of the corpora lutea, and have this to say regarding the pituitrin sensitivity of the uterus discovered by Sahako (1926) and Knaus (1929): "Although in the rabbit and possibly man, pituitrin insensitivity of the uterus may be associated with actively secreting corpora lutea, this is not true of other mammals such as the rat, mouse and probably guinea pig and cat, where there is no correlation between uterine sensitivity to post pituitary extract and functional corpora lutea." Robson & Schild (60) have also assured us that progesterone causes no decrease in the response of the cat's uterus to oxytocin. The effect of progesterone on the myometrium so well established by Reynolds has been demonstrated in the case of uterine tube contractions induced by estradiol benzoate in women in the menopause period. These were inhibited by progesterone, according to the observations of Geist, Salmon & Mintz (61). The development of the ovipositor of the bitterling has been proposed as a test for progesterone Duyvené-DeWit (62). It has been known for some time that wholly insignificant amounts of progesterone-like substances are found in the blood or urine but Holtz & Wöllpert (63) have published an



especially sensitive test for its recognition, which they state permits them to recognize the hormone in twelve cc. of blood from the second month of pregnancy or twelve cc. of urine from a newborn infant. The test depends upon the abolition of estrus produced in immature or castrated guinea pigs by the administration of progesterone. The guinea pig is stated to be thirty-three times as sensitive as the rabbit. Hertz *et al.* (64) have shown a remarkable specificity of progesterone in the induction of sexual receptivity in the theelin-conditioned ovariectomized guinea pig, as little as 0.05 international units of progesterone being thus recognizable. Lyon & Allen (65) have investigated the discrepancy in the short period of sensitivity of the rat's endometrium for deciduoma production during pregnancy, and the long period during lactation, feeling that coincident estrin is the cause of the sharp limitation of response to the early days of pregnancy. Kimura & Cornwell (66) have estimated the progesterone content of the corpora lutea of sows during normal cycles and in pregnancy. Progesterone could not be detected after the fifteenth day of the normal cycle when it is known that morphological regressive changes occur although there were increasing quantities until that time. In pregnancy it continues to increase until well into the fourth month, progesterin not being absent from the corpora lutea until the last ten days. Robson (67) has compared the vaginal changes produced by estradiol in pregnant mice with those produced by similar dosage to spayed mice treated additionally with progesterone or testosterone propionate. He feels that his results give some quantitative indication of the amount of progesterone present in the pregnant mouse—that it is comparable with a daily parenteral dose of 1.5 mg. of progesterone. Young, Boling & Rundlette (68) have shown the synergistic action of estrogen and progesterone in the induction of mounting activity in the spayed guinea pig, and the same group of investigators (69) have compared estrogen-induced heat with estrogen-progesterone induced heat in the guinea pig. The injection of progesterone after the end of an estrogen-induced heat period is followed by a second heat period, whereas this does not result if the original heat was induced by an estrogen-progesterone combination. Hensel (70) has noted that hair does not return rapidly after its removal by a depilatory agent late in the pregnancy of the guinea pig. He succeeded in imitating this phenomenon by injecting progesterone into non-pregnant

guinea pigs. On the cessation of treatment a prompt regrowth of the hair ensued. The phenomenon was not produced with estrogen or anterior lobe treatment. A most interesting aspect of progesterone relates to its relation with the adrenals. As regards normal adrenals, Callow & Parkes (1936) showed that extracts of the adrenals of some animals are rather potent in producing progestational proliferation. Beall & Reichstein (71) have recently isolated pure progesterone itself from adrenal extracts. In women with adrenal virilism Marrian has found abnormally large amounts of urinary pregnandiol—the first end-product of progesterone in human metabolism. Finally, as will be mentioned in the section on the adrenal, Gaunt, Hays, Nelson & Loomis have recently discovered the cortin-like action of progesterone in double adrenalectomy. It is interesting that Miescher, Fischer & Tschopp (72) have shown the cortin-like action of 21-oxy-progesterone (desoxycorticosterone). This substance, however, acts for only a few days, whereas some of its esters, notably the palmitate and benzoate, have been effective for three weeks.

*Metabolism of progesterone.*—An extremely important tool for the investigation of corpus luteum function in man appears to have been furnished by the discoveries of Browne & Venning, now approximately two years old but extended significantly in the period embraced by this review. It will be remembered that they showed that the urine contains a water soluble, biologically inactive breakdown product of progesterone, in the form of pregnandiol glucuronide which may be gravimetrically estimated by recovering the sodium salt. If progesterone is administered intramuscularly to a patient with an intact uterus,<sup>2</sup> from 40 to 46 percent of it may be recovered as urinary sodium pregnandiol glucuronide. This material is not naturally excreted in the follicular phase of the normal menstrual cycle, but only in the second half or luteal phase, according to Venning & Browne (73). It appears suddenly in the urine in forty-eight hours after ovulation, lasting twelve days at longest, disappearing shortly before the next menstruation, the total eliminated being in the neighborhood of 50 mg.

<sup>2</sup> Pregnandiol has not been recovered after the administration of progesterone to hysterectomized patients, but a normal proportion of administered pregnandiol itself (the sodium salt of the glucuronide) appeared in the urine. These observations appear to be the first demonstration of the essential participation of the uterus in a physiologic mechanism apart of course from its rôle in pregnancy.

and the highest daily elimination 5 to 7 mg. In the first two months of a normal pregnancy this urinary material is found in amounts comparable to amounts found in the menstrual cycle, *i.e.*, 4 to 10 mg. daily, but a rise occurs shortly after the eightieth day and a peak is reached at the eighth month, when 80 mg. daily are found. The substance disappears from the urine within twenty-four hours of delivery. Browne, Henry & Venning (74) have found that in most cases of late pregnancy toxemia, lower estrin and pregnandiol elimination were found, but state that at present a definite correlation of the amounts of urinary prolan, estrin or pregnandiol with toxemia is impossible. Weil (75) has also reported lower pregnandiol in pregnancy toxemias. The Montreal investigators prudently state that in cases of decreased urinary pregnandiol one can not possess certainty as to decrease in the production of progesterone inasmuch as we must reckon with failure of the mechanism of conjugation of pregnandiol with glucuronic acid (probably an hepatic function) as well as with failure of the renal excretory mechanism. Reports of the employment of the Montreal methods have been made by Wilson & Randall (76, 77, 78), Hamblen, Ashley & Baptist (79), and by Stover & Pratt (80). We may expect a period in which vexatious and apparently contradictory data are secured as regards the excretion of autogeneous as well as administered progesterone before all the contributory factors in this physiologic mechanism are well understood.

*Synthetic estrogens.*—Dodds (4), who is responsible for the most comprehensive studies in this realm, has published a short résumé of the field in the Singer-Polignac colloquium. Over two years have elapsed since the beginning of the long series of studies which have shown us that estrogenic activity is not necessarily related to the carbon skeleton of the naturally occurring estrogens (cyclopentanophenanthrene) and demonstrated that some relatively simple hydro-carbons have distinct estrogenic effects. It will be remembered that a contaminant of anol, still unknown in its chemical nature, was shown in 1937 to have a prodigious estrogenic potency. Robson & Schönberg (81) reported the pronounced activity of triphenylethylene—the production of full vaginal cornification in spayed mice with a one milligram dose and the persistence of effects for nine weeks when ten milligrams were employed. The material was described as effective in two hypo-

physectomized rabbits. Later Robson (82) was able to show the induction of estrous changes in the monkey and bitch by triphenylethylene and, with Bonser (83), he has recently shown that this will substitute for estrone in raising the incidence of mammary tumor in males in a strain of mice where the natural estrogen has this effect. Robson has been able to antagonize the effects of progesterone with triphenylethylene on the endometrium and myometrium of the rabbit, although estrone is three times as efficient in this respect. In 1937, Dodds & Lawson had reported the activity of several substituted derivatives of ethylene, including the hydrocarbon diphenylethylene (stilbene). Dodds, Fitzgerald & Lawson (84) then published a short table of the estrogenic activities of a number of these substances, confirming the fact that triphenylethylene is particularly potent, ten milligrams having given estrus in 100 percent of their animals. This substance was also tested by Hemmingsen & Krarup and shown to confer enhanced spontaneous activity and normal mating instincts on the ovariectomized rat. Early in 1938, Dodds, Golberg, Lawson & Robinson (85) published a note on the estrogenic activity of other synthetic materials. The paper contains the first announcement that 4, 4'-dihydroxy-alpha-beta-diethylstilbene (designated diethylstilboestrol or DAES) was 2.5 times as active as estrone. This was the first synthetic substance whose action exceeded that of estrone, being at least as potent as estradiol, since it was fully estrogenic in doses of 0.4  $\mu$ g. subcutaneously and 0.1  $\mu$ g. orally. Dodds, Lawson & Noble (86) showed that vaginal and uterine changes characteristic of estrogen were possessed by this substance, although mammary growth in the guinea pig did not seem to be stimulated as effectively as by estrone. Guldberg (14) has recently shown that DAES unfolds the same action as estrone in a castrated woman. Folley & Watson (87) have further investigated its properties which include the power to inhibit lactation in the rat and the temporary increase in the phosphatase of cows' milk accompanied by fat and non-fat solid increases. It also partially inhibits the response of the pigeon crop gland to prolactin, possibly because of depression of the bird's pituitary. Jacobsen (88) has studied the effect of DAES on the hypophysis of castrated rats and shows its estrone-like effect in causing the disappearance of castration cells and, in over-dosage, the same enlargement of the pituitary known to result from estrogens. Dodds *et al.* (89)

have not been able to further increase the estrogenic effects of this substance by esterification, but have discovered that the dipropionate and dimethyl ethers give effects lasting from fifty to one hundred and eighty days! Miescher, Scholz & Tschopp (90) show that in the case of the double esters of estradiol, the maximal prolongation of estrus occurred with the esters of the higher fatty acids, whereas Dodds *et al.* (91) have shown that this is not true of esters of stilboestrol. Noble (92, 93) has carried out a careful study of the effect of DAES on body growth and on the endocrine organs of the rat. It will be remembered that Zondek first showed (1936) that prolonged high estrone dosage impaired body growth. Noble implanted crystals of DAES in the anterior abdominal wall in amounts varying from 10 to 100 mg. It enlarged pituitaries and adrenals but atrophic testes and accessory reproductive organs were produced. Growth was seriously impaired. The pituitaries of such rats, when used as implants, were shown to contain no gonadotropic hormone. Three carcinogenic hydrocarbons, which had been employed by Haddow, Robinson, Scott & Li did not affect the pituitary or general body growth. The functional impairment of the anterior pituitary produced by DAES was marked in the features already mentioned, but Noble's studies do not appear to him to "support the view that the diuretic substance of the anterior lobe is suppressed." Dodds *et al.* (94) have uncovered another synthetic substance with estrogenic action fully comparable with that of DAES in a dihydroxy-diphenylhexadiene. Mention has already been made of the synthesis of a powerful progesterone-like substance by Inhoffen & Hohlweg (57). They have also produced an orally potent estrogenic substance—17-aethinylestradiol, the rat unit being 3  $\mu\text{g. per os}$ , whereas estradiol and estrone require approximately twenty times that dosage.

*Span of gestation and cause of onset of parturition.*—Both gonadal (estrone and progesterone) and gonadotropic substances are able to increase the span of gestation, the latter presumably because they give rise to the former. It would appear that parturition can be very considerably postponed by prolongation of luteal function. On the other hand, it has been convincingly demonstrated that a corresponding prolongation of the time of development of the fetus can not take place although a limited amount of true "over-ripeness" can undoubtedly be obtained. Fetal death with continuing pregnancy, *i.e.*, the postponement of par-

turition, appears due to impairment of placental function for reasons not adequately clear. Snyder (95) has covered the subject of the "factors concerned in duration of pregnancy" in an excellent physiological review. He stresses the fact that the term of pregnancy is a multiple of the cycle of the species; that pregnancy and parturition are under hormonal control (pituitary, placental and ovarian). By the intravenous injection of prolan into rabbits during the last quarter of pregnancy, he has been able experimentally to dissociate the decidual and muscular phases of parturition. When a new set of corpora lutea were induced on the twenty-fifth day, a premature separation of the placenta occurred in certain implantations adjacent to those still firmly adherent and destined to persist until the forty-first day (term being on the thirty-second day). Snyder thinks that the hyperemia and edema, and actual endometrial growth associated with the development of a new set of corpora, cause sufficient structural changes in some of the placentae to dislodge them. Such dislodgment is always associated with bleeding; the young may be born alive. The uterine musculature as a whole must be relatively quiescent or all would be expelled. The author rules out changes in the fetus, senility of the placenta, and mechanical distension of the uterus as causes of parturition. Bøe (96) has produced a monograph on "The study of prolonged pregnancy in rats." Though there are two forms of prolonged pregnancy: (1) due to delayed implantation and (2) due to disturbance in parturition—his paper is devoted only to the second type. Pregnant mare serum prolonged pregnancy and inhibited fetal development; frequently mummified foetuses occurred with living ones. The action of pregnant mare serum was so strikingly like that of estrone that the author considers that the secondary production of estrone played the essential, rôle in the results. Pregnancy prolan, on the other hand, acted like progesterone in that it caused the prolongation of pregnancy with fetal weight increase and the author concludes that its main effect is due to the secondary production of the other ovarian hormone—progesterone. Hypophysectomy on the sixteenth to eighteenth day disturbed the birth mechanism and prolonged gestation resulted, associated in some instances with striking increase in fetal weight. Mechanical obstruction to parturition by ligature of the uterine horns did not cause noticeable increase in fetal weights. The author has analyzed the changes in placenta,

uterus and fetus during prolonged gestation and reaches the conclusion that fetal death resulted from failure of the placenta. Bøe admits that, as yet, research has yielded little on the precise physiology of the initiation of parturition. King (97) by administering prolan from the sixth to the tenth day of pregnancy in the rat prolonged it and secured foetuses past maturity. Kirsch (98) has employed the ingenious device of surgical removal of the embryos from the uterus, the placentae remaining in situ. Regardless of the time at which this is done, the placentae are retained until the expected parturition date when they are delivered. His work clearly established that the foetus is not essential in the maintenance of gestation but it appears that the placento-uterine complex accomplishes this, possibly through the secretion of progesterone. Bunde (99) has studied the effect of Fevold's follicle stimulating hormone and luteinizing hormone on the gestation period of the rat. These substances were administered late in pregnancy, both producing heavily luteinized ovaries and both inhibiting parturition. The foetuses lived to term or longer. In other words, the results were the same as with unfractionated pituitary preparations. He also tried pregnant mare serum and found it produced large follicles and did not have as great an effect as unfractionated pituitary preparations. Highly purified progesterone preparations did not inhibit parturition in the rat even when three rabbit units per day were given from the sixteenth to the twenty-second day of gestation. Arvay (100) in his study of the factors causing prolongation of pregnancy in the rabbit, found that estrin interrupts it and while progesterone does not, he was not able to prolong gestation in the rabbit with progesterone. Prolan, however, did prolong pregnancy. He thinks it acts by lessening spontaneous activity of the uterus and reducing its reaction to posterior lobe hormone. He thinks gonadotropic hormone secretion gradually lessens and folliculin causes birth. His studies with roentgen treatment of the ovaries have led him to assign no significance in parturition to the corpus luteum hormone. After irradiation on days ten to twelve no corpora or follicles were left in the ovaries, yet pregnancy and parturition were normal. If anterior pituitary hormone was given to animals after irradiation, pregnancy was prolonged as it is in normal animals with this hormone. Tscherne (101) comments on the Arvay results to the effect that a rôle by progesterone is not



thereby eliminated since this substance can conceivably be formed in the placenta or the adrenals of the irradiated rats. Heckel & Allen (102) have shown that progesterone as late as the last four days of pregnancy in the rabbit delays and may prevent parturition. The fetuses become at most three days post maturity. The same authors originally detected the fact that estrin maintains the corpora in pseudopregnancy and now they find the same for pregnancy although damage to the placentae and embryos occurred. Much debate has centered about whether the contractions of labor are actually occasioned by secreted oxytocin but the occurrence of normal parturition after ablation of the posterior lobe of the pituitary in the rat seemed to deprive oxytocin of any physiological status as a true hormone in spite of its potent pharmacological properties. Haterius & Ferguson (103) feel they have saved the hormonal status of oxytocin by their demonstration that electrical stimulation in the region of the infundibular stalk of the rabbit shortly after parturition produces an unmistakable increase in uterine activity. Fisher, Magoun & Ranson (104) also believe that the posterior lobe hormone can not lightly be ignored as a possible instigator of parturition. They made lesions in the hypothalamus of cats which destroyed the nervous pathways to the posterior lobe and led to atrophy of the posterior lobe. In all seven operated cats, pregnancy and labor were abnormal. The authors believe that in earlier so-called complete hypophysectomies small fragments of the infundibular stem may have been left which were able to supply pitocin and oxytocin. It is, perhaps, allowable to repeat that Van Dyke has shown that a relation of progesterone to the pituitrin sensitivity of the uterus can not be established in a number of mammalian forms.

*Pregnancy tests.*—Skin tests for pregnancy have been analyzed but with the exception of the suggested use of the reaction of the African toad, *Cenopus*, (so uncommon as to be impracticable) nothing has been proposed to influence the supremacy of the very reliable Aschheim-Zondek and Friedman tests.

*Time of ovulation.*—Three years ago Burr, Hill & Allen reported the detection of ovulation in the rabbit by changes in electrical potential. This pioneer work was extended by Burr and associates (105, 106) to human beings. Studies within the last year by Burr and associates (107) and by Rock and associates (108) at Harvard confirm detection of the time of human ovulation in this way.

Rubinstein (109) has reported a temperature drop during the hours of follicular maturation and rupture. Zuck (110) also, in determining rectal temperatures in tenths of a degree, finds that this is lowest (approximately 97°F.) at mid-period (between the eleventh and the eighteenth day).

*Androgens.*—Goldberg (3) has recently summarized our knowledge of the chemistry of substances which have male hormone properties and a careful enumeration of these substances and description of their properties has been given in the *Tabulae Biologicae* (5). The list is considerable. Testosterone and its esters have been clearly established as the most potent known androgens both in power to stimulate the seminal vesicles of castrate rats or to induce growth in the capon's comb. Standardization tests have recently been summarized by Freud (6). The studies of Zimmerman (1935) and Oesting (1937) have shown that the sex hormones react with *m*-dinitrobenzene to yield a chromogen which can be measured colorimetrically and Neustadt (111) has worked out a photolorimetric method for determining urinary androgens which is said to yield "an exact calculation of the excreted amount in milligrams, international units or gamma per liter." Callow *et al.* (112) have also modified the Zimmerman method and developed a somewhat similar and highly satisfactory colorimetric method for urinary androgens. Ruzicka (1935) first suggested the comb of the newly hatched cockerel as a test object for androgens. Hamilton (113) has reported cock-like behavior including crowing in male chicks ten days after hatching, and Breneman (114, 115) five days after hatching, as a result of the administration of male hormone. Distinctions as to the effectiveness of androgens have fortunately been made in the M.E.D. and the length of effectiveness. Miescher, Fischer & Tschopp (116) have reported that testosterone-3-acetate-17-butyrate exhibits effects lasting fifty days after a single injection. Bottomley & Folley (117) injected a number of crystalline androgenic substances into immature male guinea pigs for thirty days; most of them, besides enlarging the accessories, produced marked atrophy of the testes. Moore & Price (118) have clarified the literature on the apparent elective stimulation in the rat of certain parts of the male accessory system as contrasted with others, by showing that the prostate has a lower threshold of response than the seminal vesicles and that if a sufficiently high dose of testosterone propionate is

given the normal prostate-seminal vesicle ratio can be secured. The substitution requirement for the normal growth rate of the seminal vesicles was three times the requirement for histological normality. The study reported the same grave injury to the testes of young growing males as mentioned by Bottomley & Folley. It was considered as possibly due to pituitary depression. Bottomley & Folley (119) have now studied the effects of high doses for thirty days of a very considerable number of androgens on the weights of the testes, accessory organs and endocrine glands of young male guinea pigs. Significant changes in body growth or in the weights of the hypophysis, thyroids or adrenals were produced. The absolute regression in testes weights produced by some, but not all, of the materials was viewed as acting through the pituitary, inasmuch as it was not produced by the most potent substance, testosterone propionate, if anterior pituitary gonadotropic administration was carried out concurrently. It may be said, parenthetically, that enlargement of the pituitary and depression of gonadotropic and other secretions are produced with much lower doses of estrogens than androgens. Cutuly *et al.* (120), Hamilton & Leonard (121) and others have continued to study the interesting discovery of Walsh, Cuyler & McCullagh (1934) that androgens, if given immediately after hypophysectomy, will maintain spermatogenesis in scrotal testes of hypophysectomized rats. Cutuly *et al.* (122) have been able to induce sperm head formation and ripe spermatozoa with testosterone propionate and dihydrosterone acetate when they are administered at 2 mg. daily to immature hypophysectomized rats. Recognizing that scrotal growth as well as spermatogenesis could not be produced if post-hypophysectomy regression had been allowed to take place before androgen therapy, Cutuly *et al.* had suggested that the maintenance of scrotal normality when androgens are given immediately after hypophysectomy may explain their strangely favorable effects on the seminal epithelium. Hamilton & Leonard do not believe that the effect on the scrotum is determinative inasmuch as they have already shown that scrotal retention of testes of hypophysectomized rats will not alone maintain spermatogenesis. They secured an increased weight and larger tubules in the case of cryptorchid testes when hypophysectomized animals were treated with androgens although it is admitted that seminal epithelial degeneration occurred. This phenomenon, previously known only for the rat and

not secured in the guinea pig by Scowen (123), has been demonstrated in the mouse by Nelson & Merckel (124) who have used a number of crystalline androgens, finding the most effective compound tried was androstenedione. Very recently, Nelson (125) has similarly been able to show that crystalline progesterone will preserve the seminiferous epithelium of hypophysectomized rats though the "androgenic" effects on the accessories were slight. Jacobsen & Christensen (126) have studied the action of male hormones on the seminal vesicles of castrated rats of different ages and feel that the hormone requirement for the preservation of normal histology and weight is dependent upon the size of the organs to be affected, but their experiments did not appear to justify the conclusions reached by them inasmuch as Hertz & Meyer (127) have studied the effect of surgical reduction of the amount of reacting tissue upon the quantitative effectiveness of testosterone propionate and estrone, and while there is no doubt as to direct proportionality between the final weight of seminal vesicles or prostate and the dose of androgen, or of a similar proportionality between the weight of the uterus of the immature rat and the dose of estrogen, the amount of reacting tissue in these reactions was shown to be unimportant.

*Urinary androgens.*—More than a year ago, Dingemanse, Borchartdt & Laqueur (128) indicated that there is but little difference in the daily elimination of urinary androgens by normal men and women, about 50 international units characterizing each sex, while Gallagher, Peterson, Dorfman, Kenyon & Koch (129) found rather small differences in the two sexes (50 units for women and 65 for men). Callow (130) now reports 5 to 60 units for women and 20 to 110 units for men. Both Callow and Hansen (131) have found definite amounts (a few international units only) of urinary male hormone in castrated men, a fact first announced by Bingel in 1935. It is apparent that a non-gonadal, probably adrenal, source must be admitted. Although Callow and others have indicated that only a small proportion of administered androgen is recoverable in the urine, Dorfman & Hamilton (132) would appear to have shown that castrated men can be brought to exhibit a marked increase in urinary androgen so that normal levels or higher ones (500 international units daily) are found following the administration of testosterone propionate orally or intramuscularly. Callow states that the ordinary cases of hirsutism and mild virilism in

women, including cases of Cushing's disease, are not usually associated with high urinary androgen though some instances have been found; on the other hand, adrenal tumors may exhibit very high levels, a case of 3500 international androgenic units daily occurring in a child of seven with such a tumor; from this urine crystalline dehydroandrosterone was isolated. The suspicion of adrenal tumor is always to be entertained when more than 300 daily androgenic units are encountered in the urine of a virile woman.

*Action of sex hormones on animals of opposite sex.*—The discoveries of the last few years have made it clear that androgenic hormones will, under some circumstances, unfold estrogenic properties, and the converse may be said of estrogens; some of the sex hormone substances in fact unfold male or female properties with almost equal ease. This situation makes it necessary, Korenchevsky, Dennison & Hall (56) believe, to classify sex hormones into three groups: those possessing chiefly male properties, those possessing chiefly female properties, and those bi-sexual in activity. For the latter term Parkes (133) has suggested the word "ambisexual" as preferable. He has further recommended the term "gynaecogenic" as a general term to describe activity which results in the production of the attributes of femaleness and the word "gynaecogen" to describe a substance having such activity, estrogenic and estrogen being applied only to the production of estrous changes. The capacity of many of the androgenic substances to cause growth of the uterus and mucification of the vagina would then indicate gynaecogenic activity, while transandrostenediol, which will cause actual cornification of the vagina, would also be estrogenic. Somewhat over a year ago the multiple activities of androgenic substances were dealt with rather comprehensively by Deanesly & Parkes (134). Parkes (135) has dealt with this theme in his chapter on the ambisexual activity of the gonads in the Singer-Polignac colloquium. Hill has continued his series of contributions called "Ovaries secrete male hormone." Deanesly (136) has confirmed Hill's work, using his procedure of transplantation of ovaries into the ears of castrated immature male rats. The androgenic activity of the ovaries, which was not strong, seemed to be associated with luteinization of the theca interna of the follicles in the grafts, but Desclin (137), who has produced abundant luteinization of similar ovarian grafts without improvement in the development of the prostate and seminal vesicles,

questions this rôle for lutein tissue. Papanicolaou & Faulk (138) showed that the temporal muscles of adult female guinea pigs, normally much smaller than those of males, underwent hypertrophy when treated with chorionic gonadotropin and although testosterone propionate readily produced the muscular hypertrophy in them, estrogens did not do so.

*Effect of androgens on females.*—Robson (139, 140) has shown that the estrous cycle in mice can easily be inhibited by the daily administration of 20  $\mu$ g. of testosterone or 200  $\mu$ g. of progesterone. The vaginal cornifying action produced by estradiol, estrone or triphenylethylene in spayed mice can also be inhibited by the simultaneous administration of testosterone or progesterone. The association of the male hormone with progesterone should not occasion surprise for Parkes, Korenchevsky and others have produced progestational proliferation with the male hormone. Freed, Greenhill & Soskin (141) think that because the corpora lutea are large, testosterone releases luteinizing hormone from the pituitary. Gaines *et al.* (142) report that the uteri of women, however, undergo involution on the administration of the substance. Some time ago Scipiadès (143) showed that the injection of 1 mg. of testosterone daily in the pregnant rat prevents the abortion which follows castration, but Courrier & Gras (144) have reported that the substance is powerless to prevent the interruption of pregnancy by castration in the rabbit when given in doses as high as 100 mg. daily or in cats at the 10 mg. level. They have, however, assured themselves that sufficient dosage of the substance to castrate cats which had been given folliculin creates the elaborate endometrial gland development which is so characteristic of the lutein phase of this form. Emmens (145) has tested a number of androgens in regard to their relative potency in maintaining the accessories of spayed mice. This study confirms nicely the mass of evidence now at hand which all goes to show that the order of gynecogenic potency is quite other than that of androgenic potency, trans-androstenediol being most marked in gynecogenic properties. Brooksby (146) in efforts to produce deciduomata in the rat's uterus, concludes that testosterone acts in a qualitatively different manner from either of the female sex hormones. Nathanson, Franseen & Sweeney (147), while agreeing to the complicity of the hypophysis, nevertheless feel they have demonstrated a direct effect of testosterone on both vagina and uterus. Salmon,

Walter & Geist (148) found that, when administered in adequate doses to women, atrophic changes are produced in the vaginal mucosa as judged from the disappearance of glycogen in the cells of the vaginal smear and the appearance of "deep" cells—changes referred to a suppression of the hypophysis. Birnberg, Kurzrok & Livingston (149) have shown that testosterone propionate controls the severe menopausal symptoms in women castrated by x-ray or radium and biopsies of the endometrium showed that it had not been stimulated. Phelps, Burch & Ellison (150) feel that the responses of the endometrium of spayed guinea pigs to estrone and testosterone are qualitatively the same. Zuckerman's (1937) discovery that testosterone propionate suppresses the menstrual cycle in monkeys, and stops cyclic ovarian changes, seems to be attributable to suppression of the pituitary by the male hormone inasmuch as he has recently shown that the latter does not interfere with pronounced stimulation of the monkey's ovary caused by pregnant mare serum when testosterone propionate is administered simultaneously.

*Action of estrogens on males.*—Nelson (151) has shown that whereas high dosage of estrone and estradiol will reduce the testes of normal male rats in three weeks to the atrophic picture seen in hypophysectomy, causing loss in body weight and the usual enlargement of the pituitary with chromophilic degranulation of the latter; as little as 1 mg. of androstenedione daily will counteract 1000 units of these estrogenic substances in the above effects including pituitary enlargement. It has been known for some time that androgens cannot repress the hypophysis as effectively as do estrogens. The Nelson work indicates that androstenedione has, in fact, a contrary effect on the hypophysis, and it is pointed out that the same substance mollifies the further injury to the reproductive system which estrogens occasion in hypophysectomized males. Castillo & Pinto (152, 153) have confirmed Moore & Price (1932) in the retardation of seminal vesicle atrophy after castration produced by estrone, and have shown that estrone can act synergically with testosterone propionate in further increasing the well-known hypertrophy of the male accessories which the male hormone provokes in castrates. Some anomalous effects of estrin have been found. Bokslag (154) has shown that the single administration of high doses of estrin to young normal male rats will appreciably increase the seminal vesicle weights in thirty-six



hours, and Ball (155) has shown that estrin can increase the sex activity of castrated adult male rats. Jongh, Kok & Van der Woerd (156) have shown that estrone in male dogs causes enlargement of the genitalia and especially the prostate.

*Experimental intersexuality.*—Four years ago Willier, Gallagher & Koch reported the production of intersexual changes in genetic female chicks by the injection of androgens during incubation. A considerable series of studies have now been conducted by Willier *et al.*, the last of which (157) has analyzed the biological differences in the action of four androgens—androsterone, dehydroandrosterone, androstenedione and testosterone propionate—with the clever employment of embryos with sex linked plumage characters, the genetic sex being thereby ascertained. Androsterone and dehydrosterone were shown to possess both masculinizing and feminizing effects while testosterone propionate had masculinizing effects only. At the very beginning of the Willier work, Hain (1935) reported a striking displacement of the urethra (hypospadias) in female new born rats when their mothers had received a single injection of estrone on one of the last few days of pregnancy. Shortly thereafter she secured similar changes in suckling females by the administration of massive doses of estrone to the lactating mother. The alteration of the mammalian reproductive system at so late a time had hardly been regarded as a remote possibility. Shortly after the above mentioned work, Dantschakoff began the introduction of sex hormones into the amniotic cavity of guinea pig embryos. She has also carried out extensive researches with the developing chick and in the period embraced by this review has continued to publish individual studies, as well as two notable summaries (158, 159) on the achievements and theoretic significance of experimental intersexuality. Over two years ago Greene, Burrill & Ivy began this type of work in the rat. Part of their work has been confirmed in the mouse by Raynaud (160) and in the rat by Hamilton & Gardner (161). By the administration of androgens and of estrogens to pregnant rats, Greene, Burrill & Ivy (8, 9, 10, 11, 12) have produced masculinized female and feminized male offspring. The masculinized genetic females possess uteri, oviducts and upper portion of the vagina along with epididymis, vas deferens, seminal vesicles, ejaculatory ducts, prostatic lobes, Cowper's gland and penis. The feminized males show a definite inhibition of the development of the male genital organs, possess mamillae (which are absent in normal males) and a vagina.

*Mode of administration.*—It has been well established that the free hormone—male or female—is less effective than some of the esters. Miescher *et al.* (162) have lately conducted careful studies as to the extent to which the esters of estrone and of estradiol prolong their action. Robson (163) has noted the remarkably prolonged action of estradiol benzoate butyrate. Decreased absorption rate of the administered hormones has unquestionably been one of the factors in these successes. About a year before the period to be summarized in this report, Deanesly & Parkes of London began significant work on the implantation of free crystals or tablets of hormone in the subcutaneous tissues where exceedingly slow but constant solution by the body fluids imitated to a very remarkable extent that continuous liberation of minute amounts of hormone which we must conceive to characterize the normal performance of the endocrine glands. Thus two milligrams of estrone implanted subcutaneously in brown Leghorn capons feminized the plumage more effectively than an oil solution of the most potent known ester of estrone and for a period of three months (164, 165, 166). Not only were high doses wasted as to effects, but for some reason were often poorer. Brock & Druckrey (167) used collodion bags containing estrone crystals in the peritoneal cavity of spayed rats and observed the consequent estrus continue for over a year. The method would seem to lend itself excellently to all types of experimentation where long continued, steady effects are required as in the depression of the anterior pituitary by estrogens, masculinization of females by androgens, etc. Thus in the male guinea pig, the implantation of estrone tablets of a few milligrams has led to atrophy of the testes and accessories and decreased body growth in forty days; in the rat the Leydig cells and accessories atrophy first and then the tubules. Testosterone and its propionate thus implanted in spayed rats led to a six-fold increase in uterine weight and the development of the female prostate which is rarely distinguishable [but see Witschi *et al.*, (168)] in normal females. They have suggested the clinical employment of progesterone tablets in habitual abortion. It has been known for some time that one can obtain increased effects of hormones on organs which they stimulate hematogenously if one applies the hormone locally. Lyons' device in the vaginal application of estrogens and the crop sac application of mammotropin enabled him to thus recognize exceedingly minute amounts of these substances, while McGinty *et al.* (169) have

shown that the uterine application of progesterone in immature rabbits led to the characteristic endometrial changes at one-hundredth the level required in intramuscular administration. Grumbrecht & Loeser (170) appear to have shown that the clinical intro-uterine application of estrin to an infantile hypoplastic organ will create both muscular and endometrial hyperplasia in far lower dose levels and sooner than by any other known method of dosage. It has already been pointed out that the direct application of androgens to the capon comb and that of newly hatched cockerels are in use as tests. Dessau (171) has used capon comb inunction extensively; Mühlbock (172, 173) by this method has not only shown nicely the antagonism of estradiol to testosterone but has made the striking discovery that the capon's comb can be thus further reduced by estrogens, and Deanesly & Parkes found testosterone much more effective than when administered subcutaneously. Early in 1938, Zondek called emphatic attention to the proof one can secure of the percutaneous effectiveness of estrogens and shortly thereafter the striking carefully conducted tests of Moore *et al.* (16) demonstrated conclusively that both androgens and estrogens applied to the skin as ointments or creams can be effectively absorbed. The report of Foss (174) on the successful employment of testosterone propionate in ointment form in hypogonitalism in men, heralds the adoption of these procedures in the clinic. Any marked oral effectiveness of androgens remains to be demonstrated but orally effective estrogens are well established. Greene & Ivy (174a) have shown that estriol glucuronide is ten times as effective orally as subcutaneously. The demonstration of the oral effectiveness of members of the estradiol series (*e.g.* the "aethinyl-estradiol" of Inhoffen & Hohlweg) has been paralleled, as has been noted, by the recent discovery of the oral effectiveness of a progesterone substitute (pregnen-in-one-3-ol-17) by Inhoffen & Hohlweg and Clauberg & Üstün (13) have published the first clinical report of the establishment of general uterine growth and endometrial proliferation followed by the endometrial secretion phase and subsequent menstruation through the oral administration of these two substances to an excellent test subject—a previously ovariectomized patient in whom typical genital atrophy had ensued. Guldberg (14) has reported a similar achievement in the use of stilboestrol followed by synthetic progesterone. Bishop, Boycott & Zuckerman (174b) have just published a report of

rather extensive clinical employment of stilboestrol from which it appears that the expectation that the material would play the role of a "natural" estrogen has been confirmed.

#### GONADOTROPIC HORMONE

*Brain-pituitary relationship.*—Experimental exploration of the interrelation between the hypothalamus and the pituitary is belatedly taking place. Shortly before the very recent period embraced by this review, gratifying results as concern demonstrable nervous connections of the posterior lobe with hypothalamic nuclei were reported [Ranson *et al.* (175)] but in spite of increasing certainty as to the nervous control of the anterior pituitary, it has thus far been impossible to demonstrate important nervous connections between the brain and the anterior lobe. Rasmussen (176) has studied the nerve endings in the pituitary and finds them unusually abundant in the posterior and intermediate lobes, but almost absent in the anterior lobe. In the latter they were found chiefly along the blood vessels and were probably supplying this system. Westman & Jacobsohn (177, 178, 179, 180) have been studying the gonadal effects of severing the hypophyseal stalk in rats and rabbits. If the stalk was destroyed in the female rabbit immediately after coitus, ovulation did not occur and no corpora lutea were formed, but if a period of more than two hours was allowed, then short lived corpora lutea were formed. Electrical stimulation of the brain of the rabbit, which normally causes ovulation, gave none after severance of the stalk. In the rat electrical stimulation of the vagina two to five hours after severance of the stalk resulted in pseudopregnancy so that functional corpora lutea must have been induced. The ovaries gradually become atrophic after stalk section, just as after hypophysectomy, though the pituitary remained histologically normal. The effects in the male rat are similar to those in the female, *i.e.*, rather rapid gonadal atrophy occurred. Spermatozoa disappeared—only spermatogonia and spermatocytes remained in the tubules; the seminal vesicles and prostate atrophied. Growth was limited in such rats and the thyroid sometimes gave evidence of inactivity; the adrenal was not atrophic. By implantation of the pituitaries of rats with severed stalks into immature rats, it was shown that the gonadotropic hormone had practically disappeared from the pituitary within two or three days. Brooks (181) found a normal

content of FSH and LH in the pituitaries of rabbits with cut stalks. Harris (182) found that subtotal transection of the stalk in rabbits (six) had no immediate effect except loss of sex interest and sometimes transient polyuria, but after several months the animals lost appetite, became emaciated, and died, and the gonads were found to be atrophic. A relation between hypothalamus and pituitary was also shown by the results of unipolar stimulation of the tuber cinereum of estrous rabbits; ovulation resulted just as it does after stimulation of the pituitary. Harris does not, however, consider that the impulse given the tuber cinereum spreads to the pituitary. Brooks (183) has attempted to analyze the mechanism whereby coitus excites the ovulation-producing activity of the rabbit's pituitary. Ovulation still followed coitus after complete removal of the sympathetic chain. Rabbits in which the stalk was cut, however, failed to ovulate though they mated frequently and rabbits with partially severed stalk ovulated only after many matings. (The author admits possible injury to the vascular supply due to transection.) Search for a morphological basis for the nervous control of the anterior pituitary resulted only in the detection of a few fibers resembling nerve fibers, which appeared to enter the pars anterior from the stalk. These fibers remained after sympathectomy, but not after transection of the cord, from which the author concludes that in the rabbit nervous stimulation of the pituitary occurs on mating and that the nerves ultimately involved pass down the stalk from the hypothalamus to the anterior lobe. Friedgood & Bevin (184) have found that cervical sympathectomy or superior cervical sympathetic ganglionectomy interferes with the incidence of pseudo-pregnancy after glass rod or electrical stimulation of the cervix, but not if mating with vasectomized males is allowed, from which the conclusion is drawn that there must be another important pathway to the anterior pituitary.

*Intracranial pressure and gonadotropic hormone production.*—Reports have appeared in recent years of increased gonadotropic hormone excretion in patients with increased intracranial pressure. Henderson & Rowlands (185) have investigated by the implant method the gonadotropic hormone content of the pituitaries of patients with cerebral tumors and increased intracranial pressure. They conclude that although there is an increased gonadotropic content of human pituitaries after decline of gonadal function,

there is no increase in the amount of pituitary gonadotropic hormone referable to increased intracranial pressure.

*Purification of gonadotropic hormones.*—Physical methods have predominated over chemical methods in attempts to purify the gonadotropic hormones. Chiles & Severinghaus (186) and Severinghaus, Levin & Chiles (187) have been using an improved air-driven vacuum ultracentrifuge to concentrate anterior lobe and pituitary-like hormones. They achieved marked concentration of the gonadotropic hormone in pregnant mare serum and that in castrate urine by centrifuging at 600 to 700 r.p.s. for four to six hours. No concentration was achieved of the flavianate of thyrotropic hormone by similar treatment. Spielman & Meyer (188) have used electrophoresis as a means of concentrating prolactin. They found that alcohol-precipitated prolactin migrated to the anode from pH 4.91 to pH 3.55. None was found at either anode or cathode at pH 3.31, but hormone was still present in the central chamber though decreased in potency due to the strong acid. The isoelectric point is therefore assumed to be greater than pH 3.31. Prolactin prepared by the Doisy brucine technique was found at both anode and cathode from pH 5.1 to pH 3.5; the authors therefore assume that denaturation has occurred in this process and that prolactin must consist, as postulated by Euler & Zondek, of a non-specific carrier and a specific residue which continues to be biologically active after separation from the carrier. Bernard (189) has used ultrafiltration to concentrate prolactin. Filtration was made through cellophane with pressure. A concentration of 100 to 200 fold occurs in the material left on the filter; the product is less toxic than that obtained by alcohol precipitation. The method was also useful in concentration of menopause urine. The filtration separates urinary gonadotropic hormones from estrin. Bischoff (190) has recently studied carefully the stability and chemical properties of pregnant mare serum gonadotropic hormone. Hartmann & Benz (191) have analyzed the sugar content of the hormones of the pituitary anterior lobe and of the gonadotropic hormone from pregnancy urine. There were constant amounts of carbohydrate present which could not be removed by treatment with weak alkalis or prolonged dialysis. This led them to assume the presence of glycoproteins. The nature and quantity of the carbohydrate was determined by the orcinic sulfuric acid reaction. The gonadotropic hormone of the pituitary contained

six per cent mannose, and prolactin contained nineteen per cent mannose; possibly galactose was also present.

*Standardization of gonadotropic hormones.*—There is a great deal of interest at present in methods of standardization of these hormones. A bewildering multiplicity of units has come into use and it has become increasingly difficult for the workers in one laboratory to know the amount of hormone used in experiments done in another. Levin & Tyndale (192) have proposed the mouse uterus test as the most satisfactory one for menopause urinary hormone, but admit the method is not accurate for chorionic gonadotropin or pregnant mare serum. They used tannates of the hormone which contained no estrin as judged by injection into castrate animals. They found the increase in weight of the uterus to be more sensitive and more dependable than ovarian weight. Follicular development frequently could not be seen at the dose adequate for uterine development. The mouse uterine unit is defined as the dose, which, given to five or more 21- to 23-day old mice daily for three days, followed by autopsy in 72 hours, gives 100 to 150 per cent increase in uterine weight (uterus drained). The dose doubling the uterine weight also gives vaginal canalization; the usual ovarian weight unit is five times the size of the uterine unit. Heller, Lauson & Sevringhaus (193) have used the "immature rat uterus as the assay end-point for gonadotropic substance." They find a close correlation between the uterine weight (drained) and the vaginal weight (at least at low dose levels); the minimum doses for uterine enlargement and for increase in vaginal weight were equal, and one-third to one-half as large as the minimum dose required for vaginal opening, and one-eighth as large as the minimum dose required to cause substantial increase in ovarian weight. In their opinion, vaginal opening is the least dependable end-point and uterine weight the most dependable. The uterine unit is defined as the smallest amount of hormone which produces a uterine weight above that of the maximum found in controls in at least one of three rats, or an average weight increase of fifty per cent above the average of controls. Palmer (194) sounds a note of warning against the use of the uterine unit for urinary gonadotropic hormone. At least one (sodium tungstate) and possibly other methods of gonadotropic hormone precipitation are effective in recovery of fat insoluble estrogen and are found contaminating gonadotropic hormone prepared in this way. Em-



menin is such a substance. Hamburger & Pedersen-Bjergaard (195) have been interested in the standardization of the gonadotropic principle found in the urine of pregnant women and that in the blood of pregnant mares. They gave five injections in 48 hours and autopsied after 96 to 100 hours. The unit of choice in the rat for human pregnancy urinary hormone was the estrous uterine unit. This unit was related to other units as follows: minimum for estrous uterus of the test material equalled 0.003 to 0.006 mg., estrous uterus occurred on the average at about 0.006 mg.; maximum ovarian weights were obtained at four to five times the minimum uterine dose; corpora lutea at 0.0125 mg., but irregular in appearance until sixty-four fold increase given, estrous vaginal smear occurred in eighty-six per cent of cases at 0.013 mg. From these results the authors conclude that neither the appearance of corpora lutea nor ovarian weight increases are suited to the standardization of prolan and that satisfactory estimations of prolan in rats can only be made by the uterine weight or estrous vaginal smear methods (3.2 mg. of the preparation used did not cause estrus in castrate rats). The mouse lutein unit was 0.05 mg. (or fifteen times greater than the rat vaginal unit) and was also admirably suited to the standardization of pregnancy prolan. In the case of pregnant mare serum standardization in the rat, the M.E.D. was taken as the dose which caused a perceptible increase in uterine weight (0.05 mg.). The uterine weight increase was maximum before the ovaries started to develop (0.2 mg., or four times M.E.D.). (A dose of 12.8 mg. showed no estrous effects in castrate rats.) Above this dose the ovarian weights were proportional to the dose. The appearance of corpora lutea was irregular. The M.E.D. in the mouse was slightly lower than in the rat (0.025 mg.) and the maximum uterine development occurred at sixteen M.E.D. In the mouse the ovaries began to increase in size at this dose and as the dose was further increased rose sharply in weight until eight times the control size. Corpora lutea appeared regularly at the dose which doubled ovarian weight. In a slightly later paper these authors (196) studied the effect of the method of injection on the potency of gonadotropic preparations. The method of injection did not influence the effectiveness of pregnant mare serum, but the prolan potency depended markedly on the method of injection. A single injection of prolan subcutaneously was one-fourth as active, and a single injection intravenously was one-

eighth as active as repeated subcutaneous injections (five in forty-eight hours). Marshall (197) has commented recently on methods of standardization and states that the corpus luteum unit method in mice and the vaginal smear method in rats are highly accurate for pregnancy urinary gonadotropic hormone (ratio of RU (estrous) to MU is 1:12; Danish workers' ratio is 1:15). In regard to the standardization of pregnant mare serum hormone, considering that its effect is predominantly follicle stimulating, he feels that the logical unit is the vaginal unit and he finds it most accurate. Moricard & Gothié (178) have also discussed the standardization of pregnant mare serum hormone and have constructed curves showing the relation of the logarithm of the dose to ovarian and uterine weights of mice. They propose two units: (a) the opening of the vagina in fifty per cent of mice; the five-fold multiplication in the weight of the uterus in ten mice (one  $\mu$ ); (b) constant keratinization of vagina, the fifteen-fold multiplication of uterine weight, the three-fold multiplication of ovarian weight (ten gamma).

*Specific and non-specific factors influencing the effectiveness of gonadotropic hormones.*—The subject of specific and non-specific (salts of heavy metals and colloids) augmenting agents for gonadotropic hormones is intimately related to the problem of the single or multiple nature of gonadotropic hormones and is therefore of theoretic as well as practical interest. Saunders & Cole (199) in their discussion "On the reliability of present methods for characterizing two gonadotropic hormones, follicle stimulator and luteinizer," lay great stress on the non-specific nature of augmentation resulting on recombination of hypophyseal fractions, in fact, take the position that there is not sufficient evidence at present to indicate that the so-called luteinizing fraction acts synergically with the follicle stimulating fraction in a specific way, but that on the contrary it probably acts by virtue of the same properties as are possessed by egg albumin, zinc sulphate, etc. Bischoff (200) has reinvestigated the "Factors influencing the augmentation effects produced by zinc or copper when mixed with gonadotropic extracts." He found that the optimum augmentation attainable with each salt was dependent on the pH at which precipitates were formed. He considers that both salts act merely by delaying absorption, their use being justified as less tedious than division of doses. Neither salt gave augmentation if injected separately, and

at different sites than the hormone. Chen & Van Dyke (201) found that merthiolate injected subcutaneously augments the effectiveness of anterior pituitary extract. It did not augment the action of the urine of pregnant women. The authors think it acts by a chronic inflammatory change which delays absorption.

*Factors influencing the gonadotropic content of the pituitary.*—

The effect of age and sex on the hormone content of the human pituitary as determined by implants has been investigated by Henderson & Rowlands (185), Saxton & Loeb (202), and Nürnberger (203). Hellbaum & Greep (204) have compared the "Gonad-stimulating abilities of male and female rat pituitary glands." The glands from males stimulated follicles only, except in very large doses, while those from females induced luteinization at all effective levels. Friedman & Friedman (205) have confirmed Hill that coitus leads to the depletion of the gonadotropic hormone in the rabbit's pituitary within twenty-four hours and that restitution is gradual, the return to the estrous level occurring by the tenth day after coitus. Makepeace, Weinstein & Friedman (206) compared the effect of coitus on discharge of gonadotropic hormone from the pituitary of normal (estrous) rabbits and pseudo-pregnant rabbits. In the former case ovulation and rapid depletion of gonadotropic hormone in the pituitary occurred, whereas failure of ovulation, and no such depletion of the pituitary, occurred on the ninth day of pseudopregnancy. A change in mechanism of discharge of pituitary hormone is postulated.

*Parabiosis.*—Besides methods for measuring the gonadotropic content of the pituitary by implants or extracts, methods of measuring actual gonadotropic secretion by the pituitary have been devised in the surgical union of two animals—the procedure called parabiosis. Some striking results have been reported in the last few years from such procedures and the method continues to be employed. Cutuly & Cutuly (207) have joined castrate males or females with hypophysectomized males and have observed a parallel stimulation of the spermatogenetic and interstitial elements. The physiological state of the interstitial tissue is to some extent mirrored in the condition of the scrotum, whereas the size of the testis is chiefly a measure of the degree of tubular development. When testosterone propionate or estrone was injected into castrate males or females, depression of gonadotropic activity of the pituitary occurred, preventing the stimulation of the testis of

the parabiont. Hertz & Meyer (208) have also used the parabiotic partner of the castrate to measure the gonadotropic hormone secretion of the castrate pituitary under the influence of sex hormones. On the basis of the weight of the sex organs found in the parabiont partner, the ability of androgens to inhibit gonadotropic activity is of the same order as their androgenic potency (testosterone propionate most effective and dehydro-androsterone least). Bunster & Meyer (209) find by use of the same method that the castrate male pituitary develops a higher gonadotropic potency than the castrate female. Quantitative, but no qualitative, difference was observed in the response of the pituitary to estrin.

*Effect of roentgen rays and light on pituitary.*—Grumbrecht, Keller & Loeser (210) have studied the action of roentgen rays on the structure and function of the pituitary and find no anatomical change in the pituitary of normal or castrate rats after even intensive roentgenation. The content of the pituitary in thyrotropic and gonadotropic hormone was not noticeably changed after one localized dose over the pituitary. After repeated irradiation of the whole head with toxic doses, the thyrotropic and gonadotropic hormone content of the pituitary was definitely increased. As the thyroids and gonads of the treated animal did not show increased activity, the authors conclude that the mechanism for discharge of pituitary hormones had been disturbed. Bissonnette (211) found that young ferrets could be brought into estrus 193 days before normal by "night lighting." Adult females near end of estrus, unmated and entering anestrus, could be brought back into estrus. Females, like males, became refractory to photic sex stimulation after a time and required a greater stimulus to reintroduce estrus. Cutting the optic nerve freed cycles from photic control. Hypophysectomy abolished sexual cycles (for as long as 405 days). Hartman & Smith (212) failed to increase the size of the ovaries of sterile female monkeys by irradiation of the hypophysis. Riley & Witschi (213) studied the "Comparative effects of light stimulation and stimulation of gonadotropic hormones on female sparrows." They found a poor response to both gonadotropic hormone and light in the fall; there was only a low grade response to light in late winter and spring—a time when the ovary is responsive to gonadotropic substances—and the authors state that behavioristic factors involved in nesting and mating may play the essential role in the activation of the pituitary in the breeding season. Benoit &

Ott (214) have analyzed the stimulating action of light of different wave lengths on the testicular growth of immature drakes. The red-orange part of the spectrum was the most effective testicular stimulant, blue light was least effective. Infrared showed no stimulating effect. The testes increased from five grams to a maximum of seventy grams. Benoit (215) has done some remarkable work on the effect of illumination by a quartz tube directed on the pituitary through the orbit. Though the eyeball had been removed, this also stimulated testicular development in the immature drake. In this case blue light was as effective as red. Infrared light was ineffective when applied in this manner as it had been in the normal animal. Engel (216) gave male rabbits ultraviolet irradiation for three to six weeks and found that their skins contained a substance which induced cornification of the vagina of castrate mice. He considers this phenomenon is not improbably associated with the carcinogenic activity of light, especially when it is recalled that estrus producing substances have been found in the blood of carcinoma cases as well as in cancer tissue.

*Urinary and serum gonadotropic hormones.*—Engle (217) has recently admirably summarized the differences in the biological effects of the several gonadotropic hormones. Nathanson & Fevold (218) have made the observation that post-partum urine inhibited the cycle and believe it is due, not to the lactogenic, but to the luteinizing hormone in such urine. Catchpole, Gruelich & Sollenberger (219), using tungstic acid precipitation followed by acetone-ammonia extraction to concentrate the hormone and the uterine test in 21-day-old mice as a test method, have been able to detect as little as two mouse units in a twenty-four-hour urine specimen. Boys under eleven years or under excreted less than two mouse units per twenty-four hours. Boys between twelve and sixteen years excreted two to ten units, adults ten units. D'Amour, Funk & Liverman (220) made daily gonadotropic hormone determinations during forty complete menstrual cycles. A positive response was found on the twelfth to fourteenth day in 24 out of 32 cycles. Drips & Osterberg (221) have made "An evaluation of the Frank method of determination of prolactin in the urine of non-pregnant women." They found that it is not always possible to demonstrate prolactin in the urine of normal women by this method. The time when prolactin is present varies considerably, though it is probably most often present on the thirteenth day of the cycle.

It varies in amount from five to fifteen rat units per liter, but amounts up to twenty rat units were considered normal. Amounts higher than twenty rat units may be associated with menstrual irregularities or other clinical conditions, the severity of the symptoms paralleling the amount of prolactin. Tyndale, Levin & Smith (222) have compared the "Response of normal and hypophysectomized immature rats to menopause urine injections." As previously reported, castrate urine at the same dose level produced larger ovaries in hypophysectomized than in normal animals—attributed to the more active thyroid in the latter. At high doses, castrate urine will produce some luteinized structures in hypophysectomized rats. Castrate urine and pregnancy urine will augment each other without causing luteinization.

*Hormone effects in the new born.*—Philipp (223, 224) has two papers on the hormone effects in human new born. He stresses the points that estrin, progesterone and prolactin are all formed in the placenta and are present in both maternal and fetal blood streams, so that it is not surprising that many of the changes observed in pregnant women are also found in the late fetus and the new born. Among such changes which he discusses are: (a) vaginal bleeding of new born, comparable to small amounts of bleeding which sometimes occurs in women before parturition, and due to the same cause, namely enlargement of cervix, which regresses as estrin is eliminated; (b) uterine mucosal changes in both mother and new born; (c) swelling of breasts, lactation in the mother and colostrum secretion in the new born; (d) changes in ureter in the mother, desquamation of epithelium in the ureter of the child; (e) massive desquamation of vaginal epithelium in mother and child; (f) swelling and hyperemia of vulva in both (male scrotum and whole genital region also swollen frequently); (g) adrenal and thyroid enlargement in both; (h) desquamation of skin of new born, possibly also paralleling skin and tongue changes in the mother. Fraenkel & Papanicolaou (225) have also studied the vaginal changes of new borns with reference to their hormonal origin. Sections of the vagina show high epithelium; the vaginal smears show its desquamation following delivery, and the vagina meanwhile loses its squamous character. These vaginal epithelial changes are attributed to estrogens but the gonadotropic hormone content of the blood of the newborn is reflected in the "luteinization" of their ovaries. Boling *et al.* (226) have made the striking

observation that heat responses can be elicited in newborn guinea pigs for two or three hours after birth.

*Relation between thyroid and response to gonadotropic hormone.*—

Leonard (227) finds that thyroidectomy of immature guinea pigs, as in rats, enhances the action of follicle stimulating hormone (menopause urine) but not of pregnancy urine. Thyroidectomy in chickens, he thinks, reduces their responsiveness to hypophyseal gonadotropic hormone. Tyndale & Levin (228) have examined the ovarian weight responses to menopause urine injection in normal and hypophysectomized and thyroxin-treated immature rats. They believe that menopause urine is more limited in its effect in normal rats than in hypophysectomized ones because of an inhibiting action of the thyroid, as thyroxin administered to hypophysectomized rats reduces the ovarian response to menopause urine. Müller's studies (229) on the interaction of pituitary and ovarian hormones are related to this subject. He found that hypophysectomy had a pronounced effect on sensitiveness of vaginal cornification to estradiol-benzoate, but thyroidectomy had none.

*Relation between the thymus and anterior pituitary extracts.*—

Uyldert & Freud (230) find that the thymus weight increases in hypophysectomized rats after injection with anterior pituitary extracts which are able to increase body weight and tail growth but which are almost free from gonadotropic and thyrotropic activity. In fact, they find a high correlation between thymus and body weights. They are not ready, however, to say what the relation of the thymotropic substance in their extracts is to the growth promoting effect.

*Factors controlling the functional life of the corpus luteum.*—

Robson had previously shown that corpus luteum function could be maintained by small doses of pregnancy prolan or small doses of estrone or estradiol (crystalline). He concludes from current work (231) that they act through the same mechanism. He determined that prolan would maintain corpus luteum function in rabbits hypophysectomized during pseudopregnancy, provided prolan injection was started immediately after hypophysectomy. If, however, the luteal function was maintained otherwise for a short period (three days) and the gonadotropic hormone then given, it was ineffective. He thinks this due to the decreasing sensitiveness of the ovary after hypophysectomy to the gonadotropic



stimulus, so that the ovary no longer responds with estrin production. Greep (232) has studied the "Effect of gonadotropic hormone on the persisting corpora lutea in hypophysectomized rats." In the rat the corpora lutea persist unless a dissolving factor is present. Corpora lutea were produced in the hypophysectomized rat, and hypophyseal follicle stimulating hormone or luteinizing hormone was then injected to test the effect on the duration of life of the corpora lutea. Luteinizing hormone dissolved them rapidly; follicle stimulating hormone did not—it only produced follicular growth; as estrin was produced by the new follicles, it is improbable that estrin dissolves corpora lutea. Pregnancy prolan stimulated no follicular growth in hypophysectomized rats, yet it produced prolonged estrus (fifty to eighty days) and here again the persisting corpora lutea were not injured. In fact, corpora lutea were the only ovarian structures that could have produced the estrin as no follicles were present and the interstitial tissue regressed after twenty days. Evans, Simpson & Turpeinen (233) found that lactogenic hormone would maintain pseudopregnancy in hypophysectomized rats if injected immediately after hypophysectomy and threads were inserted in the uteri five days later. Greep & Hisaw (234) have recently been able to prolong the life of corpora lutea and produce the pseudopregnant state, in a certain per cent of cases, in the rat in diestrus by electrical stimulation of the cervix. It was not possible to prolong pseudopregnancy by such stimulation.

*Ovulation.*—The gonadotropic hormone in pregnant mare serum unquestionably induces the growth of ovarian follicles and has been used effectively in hastening the onset of estrus in ewes. Davis & Kopf (235) have established the proof of the induction of ovulation in women with it. Hartman (236) has conducted over a hundred experiments on the rhesus monkey with this substance with "results far from promising," yet some experimental ovulations were induced. He stresses the fact that unduly rapid growth of follicles may be produced and a short lutein phase, as judged by the early appearance of the next menses, and prudently remarks, "The production of ovulation does not guarantee an endocrine condition favorable to the implantation of a fertilized ovum." The difficulty of estimating the correct dosage does not seem light. Foster & Fevold (237) and Foster (238) have studied the differential effects of follicle stimulating and luteinizing hormones on the juvenile rabbit in relation to follicular development and ovulation.

Follicle stimulating hormone injected subcutaneously, caused only follicular development, while it and a trace of luteinizing hormone caused spontaneous ovulation. Higher doses of the latter were less favorable, leading to atresia, cystic degeneration and luteinization. Pregnant mare serum given subcutaneously caused follicular development and intravenously caused ovulation. Follicle stimulating hormone combined with pregnant mare serum had the same results as when combined with luteinizing hormone. As has been previously mentioned in this review, Van Dyke & Li (239) have produced corpora lutea and ovulation (more frequently enclosed eggs) in cats by injection of anterior pituitary extract (fifteen to thirty rat units) or prolan (300 to 2000 rat units). The rabbit ovulating dose was only a fraction of a per cent of the cat ovulatory dose. Guthrie & Jeffers (240) have induced early ovulation in the bat by the injection of mammalian hypophyseal extract. Ovulation in this form is conditioned by the temperature and does not occur until February, but it was caused by treatment as early as December. Casida (241) brought calves to ovulation by injection of anterior lobe extracts for eleven to twelve days (subcutaneous), by pregnant mare serum for six days (subcutaneous), and by follicle stimulating hormone for two to six days (subcutaneous), followed by luteinizing hormone on the seventh day (intravenous). One to five eggs ovulated. Superovulation has been produced in the adult by subcutaneous injection of pituitary extract just before the expected heat period. Leathem (242), and Leathem & Morrell (243) have brought mature dogs in anestrus and metestrus, and immature dogs into estrus by injection of gamone (menopause urine) or Progynon-B. The latter substance had no effect on the ovary, the former caused follicular development which was followed by pseudopregnancy as in the normal cycle. Pseudopregnancy did not follow Progynon-B. Prospermin (normal human male urine) produced estrus and follicle development, the animals mated and pregnancy followed. The unitage needed was larger than in the case of menopause urine. Leonard (244), knowing that the bird's ovary does not form a corpus luteum, has been interested in finding out whether the bird's hypophysis contains LH. Confirming Witschi (245), he found that the chicken pituitary contains both follicle stimulating and luteinizing hormones. The presence of luteinizing hormone was judged by corpus luteum formation in immature rats, augmentation when injected combined with

menopause urine, and by corpus luteum formation in hypophysectomized rats and ovulation in estrous rabbits.

*Hypophysectomy—hypopituitarism: substitution therapy.*—Freud (246) has described the methods for hypophysectomy (and some of the consequences) in the rat, mouse, guinea pig, rabbit, dog and cat. Leblond & Nelson (247) have described a method for hypophysectomy in the mouse and described the histological changes following the operation which are essentially like those in the hypophysectomized rat. Parkes & Rowlands (248) have studied the effect of hypophysectomy on six immature male and two immature female ferrets. Melchionna & Moore (249) have again called attention to the frequency of occurrence of human pharyngeal pituitary fragments. Typical anterior pituitary tissue was found in fifty-one of fifty-four unselected human autopsies.

*Gonadotropic hormone in males.*—Li (250) has compared the weight response of the testes and accessories of the immature rat to prolan. He finds that the testes and epididymis increase significantly at half the dose necessary to cause significant increase in seminal vesicle, coagulatory gland and anterior and posterior prostate weights (0.02 instead of 0.04 mg.). At higher doses, however, the response of the latter group was greater and the seminal vesicles, coagulating glands and prostate were the organs of choice for quantitative work. Maxia (251) has compared the action of prolan B and A on the testes of immature rats, and though he finds the former causes hypertrophy of the interstitial tissue and not tubular development, while the latter has but a slight effect on interstitial tissue and a marked effect on tubules, he concludes nevertheless that prolan A acts like a small dose of B and that one hormone is indicated. Rubinstein (252) and Rubinstein & Radman (253) find the menopause urine (gamone), anterior pituitary sex hormone (gynantrin) and normal male urine hormone (pro-spermin) all cause enlargement of the rat testis and early descent, but not precocious maturity. Gamone resembled prolan in increasing the amount of interstitial tissue. Smith (254) has compared his experience with the post-hypophysectomy changes in the rat's testis with those occurring in the monkey. In both forms the testis reverts after hypophysectomy to the immature condition, which in the monkey means the tubules are lined chiefly by Sertoli cells, and in the rat chiefly spermatogonia. In the rat the hypophysis is not necessary for continued division of spermatogonia.

gonia. Whereas it is accepted that androgens will maintain the testes of rats for a prolonged period after operation, they do not maintain the testes of monkeys. Far more important is the fact that the testes of hypophysectomized monkeys do not respond to high doses of pregnant mare serum and sheep pituitary gonadotropic hormone. Jailer (255) has studied the mitotic index of the hyperplastic interstitial cells of the guinea pig when under the influence of pregnancy prolan and has decided there is so little division, that the increase must be extrinsic in origin (from cells which in the resting condition are undifferentiated or at least indistinguishable from connective tissue cells). Wells & Overholser (256) found that male ground squirrels hypophysectomized during the period of aspermia (July-December) responded to pregnancy urine, human male urine and pregnant mare serum hormones with descent of the testis and production of sperm. The accessories reached breeding season size. Wells (257) has studied the change in the gonadotropic hormone content of the pituitary of the male ground squirrel during this resting season (period of aspermia) and found it decreased greatly, but did not disappear. The seasonal reduction in potency could be prevented by bilateral castration. Byerly & Burrows (258) have tested the sensitiveness of the response of the new born chicken testis to pregnant mare serum. They found that a test based on the weight increase of such testes was slightly less sensitive than the test based on the weight response of the ovary of twenty-five day old rats.

*Anti-hormones.*—Many studies continue to be made relative to the conditions necessary for the production of gonadotropic anti-hormones. The specificity of anti-hormone production is still an outstanding problem, as is also the problem as to whether the prosthetic group or carrier protein produces the reaction. Chemical work has been started on the nature of the interfering substance. Collip, Selye & Williamson (259) have described the "Changes in the hypophysis and the ovaries of rats chronically treated with anterior pituitary extract." Parallel with ovarian regression, signet ring cells appeared in the pituitary. The ovaries of the adults treated with pig pituitary substance reached the peak of development sooner than did those of immature rats though the peak was higher in the young animals. The peak was delayed in hypophysectomized rats. At the height of the resistance in the normal animals, the regression of the ovaries was to be compared to that

in the hypophysectomized animals, wheel cells being present. The animals were not only resistant to the extract injected but to some extent to implants of rat pituitary. That they had developed a partial immunity to their own pituitary is also indicated by the fact that prolactin injected produced results in them similar to those in hypophysectomized rats, that is, no follicles or corpora lutea developed but "pronounced thecal luteinization." Hauptstein & Otto (260) have studied a number of the factors influencing anti-hormone production, namely, that rabbits as well as rats produced the anti-hormone to prolactin; that the anti-substance was heat stable, at least at 56°C. for two hours; that a small dose of prolactin took a longer time to produce antibodies than larger doses; that the reaction was reversible, *e.g.*, four weeks after a series of thirty-nine injections with 550 to 2200 rat units, anti-hormone had disappeared from the blood; that sex did not influence the production of antibodies; that weight of experimental animals (rabbits) was not an important factor; and that different animals produce antibodies to the same substance with differing facility, *e.g.*, antibodies to prolactin have not been detected in human beings. Hamburger (261) has determined with care the length of time necessary to produce demonstrable gonadotropic anti-hormone to pregnant mare serum in rabbits. He finds that ovaries of rabbits receiving 100 mouse units daily increased in weight for eighteen days, then decreased, gonadotropic anti-hormone being demonstrable in nineteen to thirty-two days. Mare serum accumulates in the serum of the recipients for nine to ten days, then decreases. The author believes the fall is associated with the production of inhibitory factors, even though not demonstrable at this time. Brandt & Goldhammer (262) had come to believe, on the basis of previous work, that anti-hormones are of the nature of antibodies. Anti-hormones, like antibodies, can be found in animals in which a specific physiological action cannot be detected, *e.g.*, gonadotropic antibodies are produced in very immature and in castrate animals which cannot give the typical gonadotropic response. The authors were therefore interested in determining whether the hormone, having lost its capacity to give the specific stimulus, could induce anti-hormone production. They convinced themselves that prolactin, after complete or almost incomplete loss of specific potency by heat, oxidation or short wave-lengths, could still produce anti-hormones. They think, therefore, that anti-

hormone production is dependent on a protein group which is attached to the active group, the protein still being effective antigenically after the active group is removed or destroyed. Zondek, Sulman & Hochmann (263), however, have reached the opposite conclusion from similar experiments. They found that prolán boiled one hour could still initiate anti-prolán production in spite of the fact that only 0.3 to 1.0 per cent of the active substance is left. After complete inactivation of prolán they found no anti-hormone production and the conclusion is drawn that the anti-prolán formation is due to the action of the prosthetic group and not to the carrier substance which is hormonally ineffective. Zondek & Sulman (264) believe they can selectively reactivate prolán or anti-prolán from the combination of the two by destruction of the anti-hormone by alkali, or of the hormone by acid. Rowlands (265) has studied the "Specificity of the anti-gonadotropic sera" and finds both the source and species specificity vary. Pregnant mare serum anti-serum was species specific for horse and source specific in that it neutralized the hormone from horse serum but not the horse pituitary. Human pregnancy urine hormone had a complete species specificity, but not a source specificity; it neutralized any human gonadotropic hormones from urine, serum, placenta or pituitary. It is interesting that the antibody evoked by the luteinizing substance in pregnancy urine could not neutralize the luteinizing substance of the animal pituitaries. Horse pituitary anti-serum was fully effective against both horse pituitary and pregnant mare serum, though not effective against other ungulate pituitaries. Pig pituitary was enhanced by this serum. The breakdown of the specificity of ox pituitary anti-serum extended even to species specificity. Katzman, Nelson & Doisy (266) found no anti-hormone formation in rats chronically injected with extracts of pituitaries of the same species. The equivalent of two anterior lobes were injected daily from fifty or sixty days of age. The serum was tested at intervals from 107 to 255 days. A dose of 2.1 to 3 cc. of serum was tested in immature rats with the equivalent of two pituitaries (daily for three days). Some augmentation was observed by serum taken between 105 and 133 days but no antagonism at any time. The maximum ovary weights reported occurred at 133 days; the ovaries at 255 days weighed 190 to 235 mg. Rowlands (267) has made a study of the specificity of "pro-gonadotropic sera." The augmenting sera were produced

by injection of sheep anterior pituitary extract into a castrated ram, and pig pituitary into an adult goat. The specificities of these sera were tested carefully by the rat ovary weight test and the ovulation test in rabbits. In the rat the results were as follows: the serum of sheep receiving sheep pituitary augmented sheep pituitary, but no other gonadotropic hormone, nor did it inhibit any. The serum of the goat injected with pig pituitary trebled the gonadotropic effect of the sheep extract and gave satisfactory augmentation of beef pituitary; it failed to augment gonadotropic hormone from horse pituitary, pregnant mare serum and human pregnancy urine, even inhibited them. Using the rabbit ovulation test, the author found that the serum of the sheep injected with sheep hormone failed to alter in either way the capacity of the sheep preparation to cause ovulation; the serum of the goat injected with pig pituitary inhibited the ability of pig pituitary to cause ovulation. Rowlands (268) has reported "Selective neutralization of the luteinizing activity of gonadotropic extracts of pituitary by anti-sera." Anti-sera were produced in rabbits by chronic injection of ox pituitary (supposedly predominantly luteinizing hormone). The serum was injected into rats with horse pituitary (predominantly follicle stimulating hormone, but producing corpora lutea also). This combination produced ovaries of the same size as those caused by the gonadotropic hormone alone, but only follicles were present, from which the author assumes anti-hormone formation specific for luteinizing hormone, which still allows the follicle stimulating hormone to act. Harington & Rowlands (269) have made one of the earliest efforts to fractionate anti-hormone-containing sera (anti-thyrotropic and anti-gonadotropic). The anti-gonadotropic sera were produced by daily injection of human pregnancy urine into a goat, and pregnant mare serum into a rabbit. The anti-sera were found associated with the serum proteins. The anti-gonadotropic fraction was recovered quantitatively in the globulin fraction, and was equally distributed between the pseudoglobulin and euglobulin fraction. The anti-thyrotropic activity was associated with the pseudoglobulin fraction.

*Reticulo-endothelial system.*—Wetzler-Ligeti & Wiesner (270, 271) describe what they call "Restropic effects of anterior pituitary extracts." They inject congo red and take the rate of elimination of the dye as indicative of the condition of the reticulo-endothelial



system. Some pituitary extracts stimulated its activity, and are therefore called negatively restropic. The authors are unable to identify the substance or substances affecting this system with known hypophyseal hormones.

#### HORMONAL CONTROL OF THE MAMMARY GLAND

Basic advances in our knowledge of mammary physiology in the last year can hardly be claimed. It is regrettable that the claim for the crystallization of mammotropin (prolactin) reported by White *et al.* a year ago has not as yet been confirmed, nor have other investigators produced results which have led to confirmation of the claim of Turner *et al.* that the anterior pituitary secretes another hitherto unrecognized hormone (mammogen) specifically affecting the growth of the mammary gland. Use of non-hypophysectomized animals in studies of mammary physiology led investigators to believe, (1) that nipple, duct and slight alveolar development could be induced in male and female alike by injecting estrin, (2) that simultaneous injection of estrin and progesterin caused more extensive duct and alveolar growth leading to the lobule formation typical of late pregnancy, and (3) that hypophyseal lactogenic hormone caused functional development and milk secretion in existing alveoli, whether developed under the influence of estrin alone or estrin plus progesterin. However, since in the hands of some investigators the hypophysectomized animal's mammary tree is less reactive to gonadal hormones, a controversy has grown up over the question of whether estrin and progesterin act directly or indirectly on the gland. Fredrikson (272) induced nipple and duct development with estrin and alveolar growth with estrin plus progesterin in hypophysectomized rabbits. Gardner & Van Wagenen (273) injected estrin alone (18,000 to 136,000 international units) over periods varying from nine to twenty-one weeks into male and ovariectomized female monkeys and found complete lobule formation in the mammary glands. Grant (274) found that estradiol and progesterin reconditioned the regressing mammary glands of "dried-up" guinea pigs, *i.e.*, restored them to a condition similar to that seen in the pre-parturient animal. Such animals then responded to prolactin by producing milk with a normal lactose content. Astwood (275) caused the development of abnormal mammary glands, showing cyst formation, duct dilatation, excessive epithelial proliferation and increase in peri-ductal con-

nective tissue, by injecting rats with high and prolonged doses of estrin. Astwood *et al.* (276) found that while testosterone had no effect on the extension of the duct tree of the rat gland, it did induce lobule formation. De Fremery (277) reported that under the influence of 10,000 international units of estradiol benzoate injected daily for three weeks the udders of virgin goats developed to ante-partum dimensions, and then lactated following five days of prolactin treatment. Bottomley & Folley (278) produced nipple and duct, but very little alveolar growth in male guinea pigs with androgens, especially trans-androstendiol, while Jadassohn *et al.* (279) obtained nipple growth with several androgens, estrogens, adrenosterone, corticosterone and anol applied to the nipple. The fact that these substances were absorbed systematically, and the possibility that their action may be an indirect one, was shown by the growth in some instances of the nipple contra-lateral to the treated one. Turner and coworkers (280, 281, 282, 283, 284), as has been mentioned, now believe that the pituitary not only secretes a-lactogenic substance but also mammogens instrumental in causing duct and alveolar growth. These substances are supposedly formed in the anterior pituitary under the influence of estrin or related substances. Injections of pituitaries from treated or pregnant animals into hypophysectomized guinea pigs as well as immature castrated rats, mice, guinea pigs and rabbits caused extensive arborization of ducts and proliferation of the lobule-alveolar system. Such effects have usually been attributed to the gonadal hormones, but apparently these investigators have controlled their experiments by also injecting animals with amounts of estrin expected to be in the pituitaries of animals exposed to estrin, along with pituitaries inactive as to mammogenic content. Much of the research in mammary physiology of the past year has been done with a view to learning how to inhibit rather than promote lactation. The well known fact that even small fragments of retained placental tissue may inhibit lactation has always been explained as due to placental estrin inhibiting the secretion of pituitary lactogenic hormone. Following numerous experiments on lower animals of which that of Herold (285) is the most recent, clinicians have determined the unitage of estrogenic substances necessary to inject into women during the first days of the puerperium, to inhibit lactation completely and alleviate the distressing symptoms of engorged breasts in women who have lost their

babies or refuse to suckle them (286, 287). In mice (288) as in women (289, 290) it has also been found that testosterone propionate accomplishes this same result, apparently also by altering the pituitary secretion in such a way as to decrease the necessary output of lactogenic hormone. However, Foss & Phillips (287) in commenting upon their results with Progynon-B suggest that they can be better explained on the basis of a peripheral antagonism of lactogenic hormone and estrin. Folley & Kon (291) found that 1 mg. of crystalline progesterone given intramuscularly daily for twelve days to suckling rats did not inhibit lactation. Connon (292) inhibited milk secretion in rats by injecting gonadotropin from pregnant women's urine (antuitrin-S) and found "luteinized" ovaries at necropsy. Grumbrecht & Düsterlho (293) were able to stop lactation by injecting thyrotropic hormone into parturient guinea pigs, but found that diiodotyrosene had but little effect for ten to fifteen days and then caused a slight inhibition of lactation. Considerable purification of the lactogenic hormone has been accomplished. It has been prepared free of thyrotropin, gonadotropin I and II, adrenotropin and somatotropin, and it has not been found glycotropic, glycostatic or diabetogenic (294, 295, 296, 297, 298, 299, 300, 301, 302). Folley and Young (299, 303) have found that the increase in milk production brought about by their various pituitary preparations paralleled their glycotropic rather than crop-stimulating properties, and have suggested that prolactin is not the only lactogenic substance in the pituitary. Those who desire that the term lactogenic hormone embrace all substances capable of increasing the milk output of lactating cows will probably contend that many substances, not necessarily of anterior pituitary origin and not necessarily of a complex chemical nature, also deserve the designation. Thus, thyrotropic preparations, or thyroxin, increase the milk yield in lactating cows (304, 305). It has been repeatedly shown that purified hypophyseal lactogenic hormone does not substitute for the intact pituitary body in causing or sustaining normal lactation in hypophysectomized rodents (284, 306, 307) and it is questionable whether normal lactation occurs even when thyroxin, cortin, glucose and sodium chloride are added to such treatment. But highly purified lactogenic hormone does maintain the morphologic *sine qua non* of lactation—a secreting, normal-appearing mammary alveolus—even in hypophysectomized animals. How much other pituitary

hormones synergize with the lactogenic hormone to increase or improve milk secretion (303, 308) will only be learned when investigators become willing to assay their products for the different constituents by the use of accepted standards. Riddle and co-workers (309) believe that their prolactin elevates the blood sugar, increases glycogen and fat deposition in the liver and increases the rate of heat production under certain conditions. They also find that it markedly decreases the size of the dove testis (310) due to its inhibiting action on pituitary follicle stimulating hormone. The ability of lactogenic hormone to stop the estrous cycle of rats and induce vaginal mucification (310, 311) is apparently not only due to a cessation of follicular growth but to a maintenance of functional luteal tissue (233). Ross (312) found that seven of eleven women, who by the fifth or sixth day postpartum secreted less than 400 cc. of milk, were able to nurse their infants completely after two injections of prolactin (1000 Riddle units), whereas only two of eight on 400 Riddle units and one of eight on saline were able to do so. Asimov & Krouze (313) injected 50 cc. of fresh, crude anterior pituitary extract into 510 cows and found an average increase of approximately four liters of milk over the yield observed in ninety control cows. Because most investigators have considered the crop-stimulating and mammary-stimulating or mammotropic hormones one and the same, and because the crop test as introduced by Riddle and his associates (or modifications thereof) is so readily carried out, that method has been the one of choice (298). However, it seems advisable to maintain also a mammalian standard and the lactogenic factor has, therefore, been assayed on guinea pigs and rabbits. A total dose of 1 mg. (about ten times the amount necessary to induce crop sac growth in one month old squabs in four daily subcutaneous injections) when given subcutaneously, on four successive days to virgin guinea pigs just after estrus, causes milk secretion. This is probably the easiest of the mammalian tests to carry out, but ovariectomized virgin and hysterectomized pregnant guinea pigs, and normal or ovariectomized virgin or pseudo-pregnant rabbits, all secrete milk within a few days of injection of lactogenic hormone provided the mammary alveolar units have not regressed (298, 302). Of all the anterior pituitary hormones, probably none can be detected in so minute an amount as prolactin. The use of the local crop reaction (302) allows for the detection within forty-eight hours of about

0.1 gamma of hormone, and by this method one may readily compare a standard and unknown extract. Valle (314) by making a mid-line fistula in the esophageal furrow and sealing it with a removable stopper was able to use the same pigeon many times for assay purposes. He found that forty-eight hours after injecting prolactin a positive smear could be made of the crop sac mucosa that showed evidence of epithelial proliferation and desquamation. Other workers using the local test for prolactin have assayed or compared the pituitaries of various animals in different functional and nutritional states (315, 316, 317, 318, 319, 320). Other organs and body fluids have also been tested for their content of prolactin and it has been learned that probably throughout life this substance is being eliminated continuously in the urine (321).

#### THYROTROPIC HORMONE

Although a considerable number of studies (322, 323, 324, 325) has been concerned with various aspects of the thyrotropic hormone, important advance has not been made in this field of pituitary physiology. The search for thyrotropic hormone in the urine of patients with thyroid disease is still being carried on. Cope (326) failed to get positive evidence of thyrotropic hormone in the urine of patients with myxedema but it could be detected in rabbits' urine immediately following thyroidectomy. In only two out of seventeen cases of hyperthyroidism was thyrotropic hormone found in the urine. Emerson & Cutting (327) report that the thyrotropic hormone appears in the urine regularly following thyroidectomy both in man and in animals. Its presence seems to be transient, although it was found in the urine in one patient seven months after thyroidectomy. They found it to occur only rarely in patients with hyperthyroidism or in spontaneous hypothyroidism.

#### GROWTH HORMONE

*Standardization.*—Bülbring (328) has shown that the lines of best fit, obtained when plotting gain in body weight against the logarithm of the daily dose, were parallel for two growth hormone preparations injected into hypophysectomized rats. From this data (eighteen rats) she has hazarded the assumption that all such lines will probably be parallel. The mathematical relationships which would be allowed by this assumption are obvious. Chou,

Chang, Chen & van Dyke (329) have published data indicating that a group of six normal rats is sufficient for testing growth hormone preparations in a ten-day period. However, twenty animals were considered necessary for detecting variations of one hundred per cent. After hypophysectomy, males and females responded alike. Sensitivity to growth hormone decreased between six and eight months after hypophysectomy. Both normal and hypophysectomized rats became less sensitive to growth hormone during a second series of injections as the dosage of the first series increased.

*Biological characteristics.*—Freud (330) has described the biological changes produced by the more purified fraction of growth hormone described by Dingemanse (331). It produces a gain in weight due to water in excess of nitrogen retention. Purification was shown by tests in hypophysectomized rats, but decreased potency was indicated in normal rats. It does not prevent atrophy of thyroids, adrenals, gonads and sex accessories and does not stimulate pigeon crop glands. He describes increase in tail length and weight of the thymus. Since the early studies of the Houssay school and particularly the admirable studies of Lee (333) and Gaebler (332), a relation of the growth hormone to protein metabolism may be regarded as established. Lee has shown that the best evidence at hand indicates that the anabolic action of the growth hormone is, first, to conserve and increase the so-called deposit or reserve protein, and second, to decrease the exogenous catabolism of amino acids. Mirsky & Swadesh (334) have examined this subject. They feel that the anterior pituitary can be looked upon as exerting a dual effect on protein metabolism, the first, a direct stimulation of protein catabolism in the muscles and the second, an indirect stimulation of protein anabolism through stimulation of the pancreas. They further suggest that the influence of the pituitary on growth may be dependent upon its pancreatic function.

*Purification.*—But two papers have appeared reporting continued purification of growth hormone—that of Evans, Uyei, Bartz & Simpson (336) on the use of ammonium sulfate, and that of Dingemanse (331). Dingemanse describes further purification by "adsorption" on activated charcoal in dilute alkali and its elution with phenol.

*Other hormonal factors affecting growth.*—Schooley, Riddle &

Bates (337) stress again the importance of lactogenic hormone for both body and visceral growth of hypophysectomized pigeons, but admit that prolactin mixed with other pituitary hormones has a still greater effect on bird growth. A reply to their basic contention was made by Evans (338) who showed that neither prolactin nor mixtures of prolactin and thyrotropin confer growth on the hypophysectomized mammal comparable with that given by anterior pituitary "growth" extracts lower in lactogenic and thyrotropic hormones.

*Wound repair and regeneration.*—Franseen, Brues & Richards (339) have studied the effect of hypophysectomy on restoration of liver following partial hepatectomy. The repair was somewhat slower (0.56% of cells in mitoses on the third day as compared with 0.63% in normals), but they thought it possible this was due to smaller food intake.

*Acromegaly.*—Atkinson (340) has reviewed the papers on acromegaly published from 1935 to 1937. Richet, Maranon, Pergola & Gras (341) call attention to the fact that muscular hypertrophy is associated with acromegaly and muscular weakness with deficient states of the pituitary, and assume that there must be some substance in the pituitary which controls muscle, either directly or through the adrenal. Ingle (342) has noted that hypophysectomized rats, like adrenalectomized ones, have a decreased work performance. Posterior lobe had no protective effect but one-half of the anterior lobe was sufficient to give normal performance. The improved muscle tone of hypophysectomized rats on injection of growth hormone is well known. Reed (335) has shown that myograms of the gastrocnemius muscle of white rats showed predominantly greater amplitude of maximal contraction and lesser duration after prolonged treatment with the growth factor than in controls.

*Therapy.*—Taylor (343), Lawrence & Harrison (344), Molitch & Poliakoff (345) report a total of forty-one cases of undergrowth treated with pituitary growth hormone, all of which responded. There was no evidence of carbohydrate disturbance and no evidence of a refractory phase. Beck & Suter (346) described a case of pituitary dwarfism with diabetes mellitus. The patient did not show skeletal or genital growth on establishment of an anti-diabetic régime (insulin), but responded promptly to anterior pituitary therapy.



## RELATION OF THE ENDOCRINES TO THE SKELETON

Two short reviews have been published treating experimental and clinical phases of this subject [Cohen (347) and Coryn (348)], but few original contributions of importance have appeared. A discussion of the highly important and well established relation of the parathyroid hormone to the skeleton or of the differentiating and "maturing" effects of the thyroid are both excluded from the purview of this summary. With relation to the sex hormones, Levie (349) has shown that, in contrast to growth hormone, estrin, testosterone propionate and pregnyl check the increase in length of the rat tail and growth of the tail vertebrae by causing rapid closing of the epiphyses. After castration only estrin checked the tail growth. Gardner & Pfeiffer (350) have shown that extended treatment with large amounts of estrogens causes hypercalcification of the skeleton involving a partial or complete obliteration of the marrow cavities in mice. Testosterone propionate (351) inhibits these skeletal changes. In contrast to the effect of these hormones, Freudenberger & Hachimoto (352) have shown that ovariectomy in the pregnant rat results in greater skeletal growth. The Silberbergs (353) have studied bone changes following ovariectomy in some detail. Working with guinea pigs they reported a hyperplasia and hypertrophy of the epiphyseal cartilages, but delayed ossification of the epiphyseal discs; consequently a relative increase in the size of the individual bones occurred. These effects of ovariectomy were opposed by an acid extract of cattle anterior pituitary which caused a specific growth and calcification of the epiphyseal cartilage. Pregnancy and lactation in the normal rat demonstrably stimulate skeletal and tissue growth as compared with non-bred litter mate controls [Cole & Hart (354)].

## ANTERIOR PITUITARY AND CARBOHYDRATE METABOLISM

Carbohydrate metabolism is a very complicated process involving intimate interrelations between a number of factors. While the subject is still by no means clarified, no field of metabolic research has produced as striking and important developments as the rôle of the anterior pituitary in this mechanism. Since a comprehensive and critical review of this field has been published by Russell (355) and another by Himwich (356), reference will be made only to work recently published. Long (357) has ingeniously employed the discovery of Pincus & Shapiro (358), that one can

produce a semi-diabetic state in rats by incomplete removal of the pancreas, to extend to this animal studies which he feels show that adrenalectomy ameliorates diabetes in the same way as does hypophysectomy. He seems to have shown with great definiteness that hypophyseal participation in the carbohydrate mechanism demands the presence of functional adrenal cortical tissue, and that it stimulates the production of cortin or of a different specific adrenal cortical principle presiding over carbohydrate metabolism—a principle admittedly unknown. That the hypophyseal adrenocorticotrophic hormone does increase the output of cortin was shown in the experiments of Ingle, Moon & Evans (359). Further reference to Long's work on adrenal participation in carbohydrate metabolism will be found in the subsection on adrenal cortical physiology. Emphasis should also be accorded Young's notable extension of the production of diabetes in normal intact animals by anterior pituitary extracts, a discovery originally made by Evans, Meyer, Simpson & Reichert (360) and almost immediately afterwards by Baumann & Marine (361) and by Houssay and coworkers (362). Richardson & Young (363) have convincingly shown that specific injury to the Langerhans tissue is produced in this way. Young (364) has confirmed Houssay's observation that if "diabetogenic" extracts of the anterior pituitary are not made from freshly frozen glands and kept in the cold, there are two types of hyperglycemic action, one, a true diabetogenic action which does not immediately raise the blood sugar and requires several daily injections, and the other, a loss of diabetogenic action and an immediate blood sugar raising effect. It may be that the immediate blood sugar raising action is without physiological significance, and contamination of anterior lobe extract with posterior lobe principles may account for published accounts of a rapid hyperglycemic action. Dogs proved sensitive to the diabetogenic extracts and responded to three or four daily injections of extract; however, the symptoms may disappear in four or five days in spite of the continued daily injection of the same dose of extract. Raising the dose caused reappearance of the diabetic condition. In cats and rabbits the diabetic symptoms appeared in 25 to 50 per cent of the cases, but the mouse, rat and guinea pig appeared to be almost completely insensitive. Young (365) reports studies on the fractionation of diabetogenic extracts. "Diabetogenic preparations were prepared without detectable prolactin, as

assayed by the pigeon crop-gland method . . . Moreover, prolactin prepared at low temperatures and under conditions which permit the preparation of potent diabetogenic fractions, failed to produce a diabetogenic condition when given in large doses to dogs . . . With rabbits, prolonged administration of prolactin induced, in many instances, a delayed glycosuria, ordinary prolactin being more effective than prolactin C in this respect. This glycosuria was not accompanied by ketonuria or polyuria, and a condition of diabetes could not be accurately diagnosed . . . Riddle *et al.* (1937) have recently found that in pigeons daily injections of prolactin for one to eight days increased the blood sugar level by seven to thirty-three per cent and conclude 'thus in pigeons the diabetogenic response is found to be a response to prolactin.' Again it must be emphasized that a slightly raised blood sugar level does not constitute a diabetic condition, and that in prolactin injected animals a slight emotional stimulus, such as that associated with the taking of blood samples, is very prone to provoke a hyperglycemia." The daily administration of large amounts of partially purified thyrotropic hormone to dogs failed to induce a diabetic condition. The diabetogenic factor of the anterior pituitary gland is probably a complex. Young believes the glycotropic and thyrotropic factors are possible constituents of this complex, but prolactin is not. Young (366) describes the condition of dogs rendered permanently diabetic by diabetogenic extracts as being somewhat different from depancreatized dogs, for these unoperated diabetics remain in good vigor without much loss in weight, for long periods without insulin therapy. Marks & Young (367) describe the severity of the diabetic condition as comparable with that of depancreatized dogs while on an essentially protein diet (the D/N ratio being about 3:1). When a large portion of the protein was replaced by equicaloric amounts of preformed carbohydrate, the sugar excretion was decidedly less than the amount to be expected from the preformed carbohydrate together with the ingested protein. On a diet almost entirely fat the glycosuria was practically abolished and ketonuria definitely diminished. It appears that the pancreas, though degenerated, was stimulated by the carbohydrate diet so that diabetes was ameliorated. Campbell & Best (368) by histologic examination observed extreme degeneration of the islet of Langerhans, and insulin assay on white mice showed less than two units of insulin could be extracted, whereas about

eighty units can be extracted from the pancreas of normal dogs of similar size. The acinar cells were well preserved. The observations of Young and of Campbell & Best have been confirmed by Dohan & Lukens (369). Woerner (370) injected dextrose into rabbits for two to four weeks. There was a compensatory increase in islet tissue, some hydropic degeneration of beta cells, but also some degenerative changes in the alpha cells and acinar cells. Himsworth & Scott (371) believe that they have secured direct evidence that the functional activity of the hypophysis is influenced by the composition of the diet. In hypophysectomized rabbits fed a high carbohydrate diet the injection of Young's glycotropic factor of the anterior lobe of the pituitary gland results in impairment of sugar tolerance and of insulin sensitivity similar to that observed when normal animals are given a low carbohydrate diet. Their study was suggested by the observation of Cope & Marks (372) that an extract of the anterior lobe of the hypophysis retarded, in healthy animals, the rate and degree of depression of the blood sugar after injection of insulin. Marks & Young (373) report an anterior pituitary fraction so potent that injected twenty-four hour fasted rabbits have eleven times as much liver glycogen as fasted controls. They believe it likely that the active component is identical with the "glycostatic factor" of Russell & Bennett, but it is not identical with the gonadotropic, or thyrotropic hormones. Evidence suggests it is not identical with the glycotropic factor (anti-insulin). Himsworth & Scott (374) have found Young's glycotropic factor to inhibit the fall of blood sugar accelerated by insulin in the rabbit with the liver completely excluded from the circulation; however, it does not affect the rate at which the tissues use glucose spontaneously. The action of the glycotropic factor in preventing the fall of blood sugar after insulin is independent of thyroid, hypophysis, or adrenal glands. Campbell (375) has published excellent analytical and statistical studies upon the anterior pituitary factor responsible for increasing the liver fat in fasting normal mice. It was also found that the anterior pituitary preparation produced a "glycotropic" as well as a "glycostatic" effect; for when the liver glycogen was first depleted by a period of fast (nine hours) the extract produced a simultaneous increase in liver fat and liver glycogen over the control level. Richardson & Young (376) have failed to find a significant increase of the islet-acinar ratio after injecting rats with aqueous extracts of acetone desic-

cated anterior pituitary and can not thus confirm Anselmino, Herold & Hoffman (377) as to a "pancreatropic substance."

*Specific dynamic action and the hypophysis.*—Evans, Luck, Pencharz & Stoner (378) have shown that hypophysectomy does not abolish the calorogenic action of glycine, orally administered, in rats. The metabolic rate of the hypophysectomized rat is forty to forty-five percent below that of the normal animal. Moderate injections of sodium chloride provoke a prompt increase of six to fifteen percent in the metabolic rate of the hypophysectomized rat. The respiratory quotient of the hypophysectomized rat is appreciably greater than that of the normal animal.

#### ADRENAL CORTICAL PHYSIOLOGY

The exact mode of action of the adrenal cortical hormone is still obscure, but the work of the last year has unquestionably brought us increased knowledge as to physiological mechanisms which are controlled or affected by adrenal cortical physiology, such as: the metabolism of potassium, sodium, carbohydrate and fat, the matter of phosphorylation, the regulation of body water and the circulation, and certain aspects of the function of the reproductive system.

*Corticosterone and synthetic substances tested.*—Thorn, Engel & Eisenberg (379) have tested the effect of crystalline corticosterone [Reichstein (380)] and a synthetic compound, desoxycorticosterone acetate [Steiger & Reichstein (381)], on the renal excretion of electrolytes in normal and adrenalectomized dogs. The sodium- and chloride-retaining effect of these crystalline substances parallels their effectiveness in maintaining the life and well-being of adrenalectomized dogs. The synthetic compound appeared to be much more active in its sodium- and chloride-retaining effect than the crystalline compound obtained from the adrenal cortex. All of these compounds produced a potassium diuresis in normal dogs. These authors feel that in the adrenalectomized dog maintained under constant metabolic conditions, the renal excretion of electrolyte furnishes an extremely sensitive index of adequate therapy. Within twenty-four hours after the reduction or withdrawal of an active preparation, a marked diuresis occurs, associated with a considerable loss of sodium and chloride. This change usually precedes the rise in blood non-protein nitrogen and occurs while the animal is still apparently in good condition. The contention that

potassium is the important factor in adrenal cortical function rather than sodium may be said to have gained considerable ground. Marenzi (382) suggests that the adrenal cortical hormone is a factor necessary for: (1) binding potassium to tissues, especially muscles, (2) regulating the equilibrium of potassium between tissues and plasma, and (3) simultaneously regulating the renal excretion of potassium. In contrast to this epinephrine, he believes, has to do with the rapid mobilization of liver potassium.

*Muscular intra-cellular potassium.*—Harrison & Darrow (383) have found that the concentration of potassium in the muscle cells is constantly increased in rats showing symptoms of adrenal insufficiency whereas in animals cured of adrenal insufficiency either by adrenal cortical hormone or by hypertonic solutions of sodium chloride and sodium bicarbonate, the concentration of potassium in the muscle cells is identical with that of normal controls. No changes in the potassium content of the liver cells are found. They suggest that the adrenal cortical hormone may have to do with the regulation of the concentration of potassium within the cell and the control of the concentration of sodium and other solutes in the extracellular water. Removal of the adrenals in cats with diabetes insipidus from hypothalamic injury produces no constant or marked decrease in the serum sodium or chloride, but there is the usual increase in the concentration of serum potassium as shown by Winter, Gross & Ingram (384). External symptoms of adrenal insufficiency are the same in the doubly operated animals as in those with only the adrenals removed, except that the symptoms develop more rapidly in the former group and the animals do not survive as long as do those with only the adrenals removed. This would suggest that the changes in sodium are less characteristic of adrenal insufficiency than are disturbances in potassium metabolism or distribution.

*Interrelation with posterior lobe.*—An interrelationship between the posterior pituitary and the adrenal cortex as far as salt metabolism or distribution is concerned has been postulated by Silvette & Britton (385); by Winter, Gross & Ingram (384); and by Gaunt, Remington & Schweizer (386). Karlson & Norberg (387), and Debré, Marie, Nachmansohn & Bernard (388) have presented evidence which indicates that in diabetes insipidus there is a diminished ability to concentrate sodium and chloride in the urine. This is consistent with the findings of Winter *et al.* (384)

reported above, that the sodium and chloride levels in the serum of cats with both diabetes insipidus and severe adrenal insufficiency are normal. Silvette & Britton (385) have suggested that the adrenal cortical hormone acts as a physiological antagonist to the anti-diuretic principle of the posterior pituitary in its influence upon the excretion of water and sodium chloride. A disturbance in water balance accompanies all conditions following adrenalectomy. Uylert (389) has shown that in normal dogs forty-five percent of the daily fluid intake is excreted by the kidney. When normal dogs are given 0.3 gm. sodium chloride per kilogram body weight, the water excretion is sixty-four percent of the intake. In the case of incipient adrenal insufficiency treated with cortin, the water excreted amounts to fifty-eight to sixty-eight percent of the intake. If the cortin is withdrawn for a few days, the dogs go into a negative water balance. Sodium chloride increases water retention in adrenalectomized dogs. Gaunt, Potts & Loomis (390) have shown that there is a lowered ratio of water intake to urine output in all conditions following adrenalectomy, and whether or not a diuresis is present depends upon the water intake. This is in keeping with the observations of Ingram & Winter (391), namely, that the removal of the adrenals from cats with diabetes insipidus results in a rapid fall in urine output due largely to the suppression of fluid intake. The reduced survival time of these diabetes insipidus cats after the adrenals have been removed is not due primarily to the negative water balance which occurs, since the withholding of fluid from diabetes insipidus cats in which the adrenals are intact, causes a more pronounced negative water balance without fatality.

*Relation to carbohydrate metabolism.*—That the adrenal cortex sustains a relation to carbohydrate metabolism seems to be definitely established. Long & Katzin (392) and Russell & Craig (393) have shown independently that the injection of adequate amounts of adrenal cortical hormone (e.g., 1 cc. of Upjohn's adrenal cortical extract or 0.03 mg. of Kendall's crystalline compound-B every hour for twenty-four hours) will not only prevent the depletion of carbohydrate stores of fasted hypophysectomized rats but will also restore them after they have been depleted by fasting. The blood sugar and liver glycogen values in the experimental rats exceeded the fasting levels of normal rats. Long & Katzin (392) have also shown that in these cortin-treated, hypophysectomized



rats there is an increased nitrogen excretion and a marked improvement in their physical condition. Long (394) suggests two possible explanations for the increase in liver glycogen brought about by cortin: (1) the hormone may stimulate increased production of glucose from protein, or (2) the cortical hormone may decrease the rate of glucose utilization in the tissues, which leads to an accumulation of liver glycogen. Long, Fry & Thompson (395) have shown that glycosuria could be made to reappear in depancreatized-adrenalectomized rats by the injection of large amounts of the purified adrenal cortical extract. The administration of sodium chloride was ineffective in restoring glycosuria in these doubly operated animals. By injection of the same extract, glycosuria was produced in normal rats (396). Himwich, Fazekas & Martin (397) have produced a chronic adrenal insufficiency in depancreatized dogs and cats by ligating the lumbo-adrenal veins on both sides. The adrenal insufficiency was not sufficiently severe to cause disturbance in serum total base and yet there was amelioration of the diabetes mellitus, as evidenced by prolongation of life, decrease in glucose, ketone and nitrogen excretion and decrease in the D/N ratio. They conclude that the maintenance of a normal electrolyte pattern is not directly related to the influence of adrenal cortical hormone upon carbohydrate metabolism. The relation of adrenal cortical hormone to carbohydrate metabolism is apparently quite distinct from the glycostatic function of the anterior pituitary, as shown by Bennett (301). He found that the muscle glycogen in the fasted adrenalectomized rat could be maintained within normal limits by cortin and sodium chloride, but that this was without effect in hypophysectomized rats. On the other hand, anterior pituitary extract maintained the muscle glycogen in the fasted hypophysectomized rat in the absence of both adrenals.

*Interrelation of electrolyte and carbohydrate metabolism.*—The relation of potassium to carbohydrate metabolism has been studied by Silvette, Britton & Kline (398, 399) and by Kendall (400). The former showed that the injection of subtoxic doses of potassium salts into rats resulted in a marked rise in blood sugar and a decrease in the liver, muscle and cardiac glycogen. When potassium and glucose were injected simultaneously, the blood sugar level was raised one hundred percent over that of the controls. The potassium seemed to suppress the deposition of glycogen in

the liver and muscle. Kendall (400) has presented very interesting data on the influence of cortin, insulin and glucose on the metabolism of potassium. He found that massive doses of cortin to normal rats produced but a transient rise in the rate of excretion of potassium and no significant retention of sodium. On the other hand, rats which had been partially depancreatized showed a ten-fold increase in potassium excretion upon the administration of cortin. Partially depancreatized rats do not always show a spontaneous glycosuria; however, when cortin is given to these animals a glycosuria develops. Accompanying the increased excretion of potassium and glucose there is a marked decrease in body weight. The excess loss of potassium is not directly associated with the severity of the glycosuria. For instance, there may be little or no glycosuria in some cases in which the loss in body weight and the increased potassium excretion are striking. The administration of potassium chloride to partially depancreatized rats causes an increased excretion of glucose. This would seem to indicate that the increase in the excretion of glucose is primarily due to a change in the distribution of potassium within the tissues. Another very important observation in these partially depancreatized rats given cortin was the marked diuresis which accompanied the excessive excretion of potassium and the loss of body weight. These data give further support to the hypothesis that the adrenal cortical function is intimately related to the metabolism of potassium which in turn has a profound effect on carbohydrate metabolism. In a very recent study by Kendall, Flock, Bollman & Mann (401, 402) on the influence of carbohydrate metabolism, adrenal cortical hormone and sodium chloride on potassium metabolism during intravenous injection of ten percent glucose into adrenalectomized dogs, it was found that only the concentration of potassium could be correlated with symptoms of adrenal insufficiency.

*Fat metabolism.*—MacKay and his group have extended their studies on the relation of the adrenal cortex to fat metabolism. MacKay & Carne (403) have shown that adrenalectomy reduces the deposition of fat in the regenerating liver tissue of fasting rats, just as it interferes with fat transport to the liver in other conditions, such as in phosphorous poisoning. In almost every condition in which adrenalectomy prevents the transport of fat to the liver, the administration of glucose does likewise, provided the glucose is absorbed and can be utilized. Yeakel & Blanchard

(404) found that the phospholipids and fatty acids of the blood of cats decreased after adrenalectomy. MacKay & Barnes (405) have shown that adrenalectomy increases the rate of removal of acetoacetic acid and beta-hydroxybutyric acid from fasting rats fed these substances. They conclude that in the absence of the adrenals, there is a small but consistent increase in the percentage of acetoacetic and beta-hydroxybutyric acids destroyed by oxidation or other metabolic processes. Since the fasting adrenalectomized rat has a low blood sugar, almost no liver glycogen and a deficient formation of glucose from lactic acid or protein sources, it may be that the ketones are burned for available quick energy. However, Mirsky (406) suggests that the diminished ketonuria occurring after adrenalectomy is due to an increased renal threshold for ketone bodies.

*Relation to muscle physiology.*—The relation of adrenal cortical hormone to muscle physiology has been investigated by several groups of workers. Hall & Müller (407) could detect no significant changes in the spontaneous activity of normal rats after administration of fairly large doses of cortin over periods of two to three weeks. Hitchcock, Grubb & Hartman (408) have studied the effect of adrenal cortical hormone on the oxygen consumption of normal human beings in relation to work capacity. While the hormone produced no significant effect on basal oxygen consumption, it depressed the oxygen consumed while the subjects were standing erect, as compared with the uninjected controls. There was a still greater depression of oxygen consumption while the subjects were walking on a treadmill at the rate of 100 meters per minute. Missiuro, Dill & Edwards (409) found that the efficiency with which easy walking was performed was increased for some days after the period of injection. The capacity for anaerobic work was not increased; the only notable effect in moderate or severe work was in the blood pressure during recovery; this was lower in all the early stages of recovery and the resting value was reached sooner. From their data on respiratory quotients there was no evidence of a greater preferential utilization of carbohydrate after cortin. Ingle (410, 411) has shown that cortin increases the work capacity of adrenalectomized and hypophysectomized rats. The mechanism by which cortin affects muscular activity has not been given a great deal of attention. Tipton (412) found that adrenal cortical hormone had a slightly depressing effect upon the po-

tassium loss during muscle stimulation; it had no significant effect on the recovery of muscles subsequent to stimulation.

*Relation to heat production.*—The relation of the adrenal cortex to heat production has been studied by Horvath, Hitchcock & Hartman (413). They found that the basal heat production in rats was unchanged after unilateral adrenalectomy; however, when rats with only one adrenal had been exposed to an environmental temperature of 4°C. for a time, the heat production measured at 29°C. was not elevated to the same extent as that of normal animals. The unilateral adrenalectomized animal showed only seven percent increase in heat production as compared with twenty-two percent in the intact controls. Furthermore, at an environmental temperature of 4°C. the maximum heat production of unilateral adrenalectomized rats is less than that of normal animals, and the response occurs more slowly. Horvath (414) found that doubly adrenalectomized rats showed an actual decrease of ten percent in heat production under the same experimental conditions. Ring's (415) findings do not confirm these observations. He found that removing most of the adrenal cortex does not decrease the stimulating effect of cold. His conclusions are that complete adrenalectomy depresses many living processes, and oxidation secondarily, and that the adrenal cortex has nothing to do with the "master" reaction, for oxidation at the temperatures studied.

*Relation to the circulation.*—Swingle and his collaborators (416, 417) have contributed additional support to their hypothesis that the adrenal cortical hormone is essential for fluid exchange between vascular and extravascular tissues, and the maintenance of the circulation. This effect, they feel, is due to its function of regulating capillary tone. They have shown that adequate doses of adrenal cortical hormone prevent the fall of blood pressure and circulatory collapse in animals given massive doses of adrenalin or other shock inducing procedures.

*Relation to hypertension.*—The work on experimental hypertension seems to have some bearing on the problem of capillary tonus. Goldblatt (418) has shown that bilateral adrenalectomy prevents the development of hypertension which is produced by the constriction of the renal artery, and conversely, that the hypertension induced in this way is not maintained if the adrenals are removed. In the last year Page (419) has confirmed this ob-

servation. His interpretation of this finding is that the relation of the adrenal cortex to this condition is chiefly to maintain the body in such a state that it can respond to the constriction of the renal artery by developing arterial hypertension. We must gather from this that he does not consider that the adrenal cortical hormone is involved specifically in developing this type of hypertension. Collins & Wood (420), who have confirmed the above finding, accept the same view.

*Phosphorylation.*—This hypothesis set forth by Verzá, which has been reviewed in the last two volumes of the *Annual Review of Biochemistry* (1937, 1938), ascribing to the adrenal cortical hormone an important function in the process of phosphorylation, correlates many of the functions of the adrenal cortex suggested by other workers. Verzá (421) has summarized his view in the abstracts of the XVI<sup>th</sup> International Physiological Congress, August, 1938 (Zürich). This very important contention as to adrenal cortical physiology deserves more attention from other investigators than it has thus far received. In the last year the only important contribution to the Verzá view is that of Pijoan & Oberg (422) who have confirmed Verzá & Laszt (423) in that adrenalectomized animals can be maintained in good condition with flavin phosphate but that cortin can not maintain the life of the adrenalectomized rat on a flavin-free diet.

*Cortin-like action of sex hormone.*—Further studies have been carried out on the cortin-like action of the sex hormones, supporting the earlier work of Thorn & Harrop (424). Thorn & Engel (425) have shown that progesterone, estrone, estradiol and testosterone propionate injected into normal dogs cause a decreased excretion of sodium and chloride by the kidneys and a slight increase in the excretion of potassium. However, none of these substances except progesterone had any effect on prolonging the life of adrenalectomized animals. Gaunt & Hays (426, 427) have shown that progesterone will maintain adrenalectomized ferrets in excellent health, whereas the estrogenic hormones are toxic to adrenalectomized ferrets. Gaunt, Nelson & Loomis (428) have shown the same effect in adrenalectomized rats, although they required ten times as much as the ferret. Koch (429) and his collaborators found that testosterone propionate when administered to eunuchoids caused a decline in the urinary excretion of sodium and chloride, a slight decline in the excretion of potassium

and a retention of nitrogen, with the blood non-protein nitrogen remaining unchanged.

*Cushing's disease.*—Extracts of the blood and urine of patients with Cushing's disease, prepared by a method used for extracting adrenal cortical hormone from adrenal tissue, have been shown by Anderson & Haymaker (430) to prolong the lives of adrenalectomized rats beyond the survival period of untreated controls. The cortin effect of 1 cc. of blood was equivalent to four to six grams of fresh adrenal tissue. It was estimated that a twenty-four hour urine specimen contained the equivalent of 400 gm. of adrenal tissue. The sodium and potassium levels in the serum and the urinary excretion of these electrolytes in patients with Cushing's disease showed a shift from the normal which is diametrically opposite that found in adrenal insufficiency. From these studies Anderson, Haymaker & Joseph (431) have suggested the hypothesis that Cushing's syndrome is produced by an excess of adrenal cortical hormone (cortin).

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INSTITUTE OF EXPERIMENTAL BIOLOGY  
UNIVERSITY OF CALIFORNIA  
BERKELEY, CALIFORNIA

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